CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

204629Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

From: Timothy J. McGovern, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 204629
Agency receipt date: March 5, 2013
Drug: Jardiance® (empagliflozin)
Sponsor: Boehringer Ingelheim

Indication: Adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments: The pharmacology/toxicology reviewer and supervisor concluded that the nonclinical data support approval of empagliflozin for the indication listed above.

The recommended pharmacologic class for empagliflozin is a sodium glucose co-transporter 2 (SGLT2) inhibitor. Empagliflozin would be a second-in-class SGLT2 inhibitor; canagliflozin was previously approved.

A complete nonclinical program was conducted by the sponsor to support approval of empagliflozin. Empagliflozin elicited expected pharmacological responses in the species evaluated. The characterized toxicity profile relates to a great degree to the pharmacological response. Key findings included renal effects, disruption of calcium homeostasis, and increased incidence of neoplasms (renal tubular carcinoma and adenoma, testicular Leydig cell tumors, and hemangiomas). In all cases, the findings were either of relatively low severity, associated with a no-observed-adverse-effect-level providing acceptable exposure margins, or not identified during the clinical program. In all, they were not considered to represent significant clinical safety risks. Empagliflozin tested negatively in a battery of genetic toxicity studies.

Although the battery of reproductive toxicity studies did not identify teratogenic effects, SGLT2 inhibitors may present a potential risk to renal development and maturation based on findings in juvenile rat toxicology studies. The division informed the sponsor at the pre-NDA meeting that empagliflozin shares this risk and that appropriate language should be included in the label. The division included this language in the proposed label and recommended listing the product as a "Pregnancy Category C", consistent with the current label for canagliflozin.

Conclusion:
I agree with the division pharm/tox conclusion that empagliflozin can be approved from the pharm/tox perspective. Listing the drug as a "Pregnancy Category C" and incorporating language in the label consistent with that for the current label of canagliflozin appears appropriate.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

TIMOTHY J MCGOVERN
03/04/2014

Memorandum

Pharmacology/Toxicology
Center for Drug Evaluation and Research
Division of Metabolic & Endocrine Products

NDA SECONDARY REVIEW

Date:	07 Nov 2013
NDA #	204629
Sponsor:	Boehringer Ingelheim Pharmaceuticals
Drug:	Empagliflozin (SGLT2 inhibitor)
Primary Reviewer:	Mukesh Summan, Ph.D., DABT
Secondary Reviewer:	Todd Bourcier, Ph.D.

Boehringer Ingelheim (BI) is seeking market approval for empagliflozin, proposed trade name Jardiance, as a treatment option for Type 2 diabetes. Empagliflozin is a small molecule inhibitor of the sodium glucose co-transporter 2 (SGLT2), a protein predominately expressed by the renal proximal tubule epithelium that serves as the primary mechanism by which the kidneys reabsorb filtered glucose. Inhibition of its function by empagliflozin results in substantial loss of filtered glucose to the urine in an amount proportional to the glomerular filtration rate and the predominating plasma glucose level. The urinary loss of filtered glucose by inhibition of SGLT2 is sufficient to reduce elevated plasma glucose levels present in type 2 diabetics. Empagliflozin would be a second-in-class SGLT2 inhibitor, should it be approved.

Dr. Summan, the primary nonclinical reviewer, concludes that the pharmacology and toxicology data support approval of empagliflozin (25mg qd). *I concur with Dr. Summan's assessment.*

Empagliflozin elicits the expected pharmacological response from inhibiting SGLT2 in the species used for toxicological assessment, typified by glucosuria, polyuria, and electrolyte changes. Higher doses elicit responses expected from inhibiting SGLT1 in the gut including carbohydrate malabsorption and gastrointestinal disturbances. Much of the toxicological profile defined in rats, mice, and dogs reasonably relate to the pharmacological response which was exaggerated at the higher exposures tested in the toxicology studies. Note that the clinical dose decreased from 50mg to 25 and possibly 10mg/day after most nonclinical studies were completed, which correspondingly increased the multiples of clinical exposure that were toxicologically assessed.

The following summarizes key issues that arose during review of the nonclinical program with empagliflozin.

Renal Health

Consistent with the drug class, rodents were more sensitive than dogs regarding renal histology after chronic exposure to empagliflozin. Exposure at the lowest doses tested in rats and mice approximated 2- to 5-times clinical exposure, and resulted in mineralization of the tubules and

papilla and dilatation of the renal pelvis, ureter, and bladder. These histological changes were not severe and are considered a consequence of (or adaptive change to) chronic glucosuria/polyuria and calciuria observed with this class of drugs. Exposure at the lowest dose tested in dogs (10mg/kg) was approximately 12- to 19-times higher than clinical exposure, and resulted in no adverse change to renal histology despite evidence of pharmacological activity (glucosuria/polyuria) in the 52-week chronic toxicology study. Serum BUN and creatinine were not significantly changed, consistent with a lack of apparent adverse renal histology at this exposure level.

Administering higher doses of empagliflozin elicited adverse renal pathology in rats, mice, and dogs to differing extents. Least severe findings were reported in rats, marked by tubule dilatation and vacuolation with lipid inclusions, and tubule basophilia and hyperplasia. More severe findings were reported in dogs and mice. In dogs, chronic interstitial nephritis, tubular nephropathy with fibrosis and degeneration occurred at 100mg/kg (~220x MRHD), oddly without any change in mineralization or markers of tubule injury (NAG and urinary GST). Such renal pathology in dogs is not typical for the drug class, and may reflect toxicology specific to empagliflozin. In mice, chronic nephrotoxicity was evident at 1000 mg/kg (45x MRHD), marked by single cell necrosis, karyomegaly, hypertrophy/atrophy, and atypical hyperplasia of the renal tubules, culminating in renal tubule adenoma/carcinoma in males. The lower 300 mg/kg group showed tubular injury but at lesser severity than the 1000 mg/kg group. Given the high exposure at which these toxicities were observed, they are considered of little risk to human subjects with normal renal function taking 25mg empagliflozin per day.

Results at the lowest exposures in rats (≤5x clinical dose) and dogs (≤19x) indicate that empagliflozin would not be a renal toxicant in human subjects at the clinical dose of 25mg per day, taken chronically. There remains a risk of urinary tract infections/pyelonephritis secondary to chronic glucosuria, which is a recognized risk in this and other programs for SGLT2 inhibitors.

Tissue Mineralization and Bone Health

A recurring finding with SGLT2 inhibitors, empagliflozin included, is disruption of calcium homeostasis in rodents and, to a lesser degree, in non-rodents. The calcium disruption typically manifests in rats as trabecular bone accretion, calcification of soft tissues, hypercalciuria, and complex changes in bone biomarkers. Consistently, serum (1,25)-dihydroxy vitamin D and parathyroid hormone drastically decline. In non-rodents, less severe changes in biomarkers are reported and bone histology does not change appreciably over the course of chronic toxicology studies. Evidence exists for both carbohydrate malabsorption (secondary to inhibition of intestinal SGLT1) and calcium/phosphate loss via osmotic diuresis as contributors to the calcium imbalance.

Empagliflozin was atypical in that consequences of disrupted calcium homeostasis were only observed in the 2yr rat study. Increased trabecular bone and extensive vascular mineralization in multiple organs was observed at doses ≥300mg/kg and most notably at 700mg/kg. A very late submission by the sponsor reported hypercalciuria in rats, consistent with other SGLT2 inhibitors. Hypercalciuria and reduced parathyroid hormone was also reported (late) in mice, but extensive tissue mineralization was not observed despite a 2yr dosing duration. The long 2yr duration of exposure required to first detect bone accretion and soft tissue/vascular mineralization in rats lessens confidence that the lack of such in the 1 year dog study is sufficient evidence of absence. Clinical monitoring for this particular toxicity was done in part by

collecting markers of calcium homeostasis in the course of clinical trials, including serum calcium, parathyroid hormone, and vitamin D isoforms. According to the reviewing medical officer, there was no evidence that empagliflozin changed these biomarkers to any appreciable extent in clinical trials. Therefore, this spectrum of toxicology observed in the nonclinical studies presents a low risk to human subjects at the proposed dose of empagliflozin.

Carcinogenicity

Four of five investigational SGLT2 inhibitors that have filed final or interim findings from rodent carcinogenicity studies with the Division have reported neoplasms of the renal tubules, adrenal gland, or testicular Leydig cells. The single agent that did not observe tumors in these tissues nonetheless reported an increased incidence of atypical hyperplasia of the renal tubules. The tumor response is variably observed in mice and rats, but more commonly in rats.

Consistent with the class, empagliflozin increased the incidence of renal tubular carcinoma and adenoma in male mice most convincingly at a dose of 1000mg/kg (45-times clinical dose). The sponsor recently submitted an unsolicited and rather substantial 'mode-of-action' paper aimed at explaining the cause of the renal tumors, which cannot be fully reviewed within the remaining timelines of this cycle. However, parsing the relative contribution of nephrotoxicity observed at this dose (from whatever cause) versus carbohydrate malabsorption is unnecessary to conclude a minimal clinical risk based on the satisfactory ~11x safety margin to the NOAEL.

Empagliflozin also numerically increased testicular Leydig cell tumors in male rats, an effect that appears to be related to the drug class. The clinical risk is considered low based on the 2x safety margin relative to the NOAEL, the much greater sensitivity of rats vs. humans to this particular tumor type and mode of action, and the observation that LH/testosterone levels have not been altered by SGLT2 inhibitors in clinical trials.

Empagliflozin is the first SGLT2 inhibitor to increase the incidence of hemangioma at a high dose, specifically in the mesenteric lymph nodes of male rats. Of note, evidence of lymphadentitis was also present at this dose level. The cause of lymphadentitis is uncertain, but is plausibly related to the increased intestinal dilatation and glandular stomach discoloration observed in this dose group. The clinical risk is considered low based on 1) the small increase relative to the control group, and 2) the high 42x exposure multiple at the dose associated with the increased tumor incidence.

Pregnancy and Lactation

The sponsor was informed at the pre-NDA meeting in November 2012 that SGLT2 inhibitors may present a potential risk to renal development and maturation based on findings in juvenile rat toxicology studies. Due to differences in timing of kidney development/maturation between rats and humans[1], these adverse effects seen in the kidneys of juvenile rats are considered relevant to the assessment or reproductive and developmental risk for communication in the drug label. The sponsor was informed that empagliflozin shares this risk due to its documented presence in fetal tissues and maternal milk in rats, and that appropriate draft language should be conveyed in the label. (b) (4) Therefore, the reviewer and I recommend that empagliflozin be listed as 'pregnancy category C', that appropriate alternative therapies be considered during pregnancy especially during the second and third trimesters, and that a decision should be made whether to

[1] Suzuki, M (2009) J Toxicol Sci 34;SP267-271 *and* Zoetis T and Hurtt ME (2003) Birth Defects Res 68;111-120

discontinue nursing or to discontinue empagliflozin, taking into account the importance of the drug to the mother. This language is consistent with the current drug label for canagliflozin, a currently marketed SGLT2 inhibitor.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

TODD M BOURCIER
11/07/2013
pharm/tox supports AP

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 204629
Supporting document/s:	0000/0001
Applicant's letter date:	March 5th 2013
CDER stamp date:	March 5th 2013
Product:	Empagliflozin (BI 10773 or BI 10773 XX)
Indication:	Type 2 Diabetes Mellitus
Applicant:	Boehringer Ingelheim Pharmaceuticals Inc.
Review Division:	DMEP
Reviewer:	Mukesh Summan, PhD, DABT
Supervisor/Team Leader:	Todd Bourcier, PhD
Division Director:	Jean-Marc Guettier, MD
Project Manager:	Pat Madara

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 204629 are owned by Boehringer Ingelheim Pharmaceuticals Inc., (BIPI) or are data for which BIPI has obtained a written right of reference. Any information or data necessary for approval of NDA 204629 that BIPI does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 204629.

TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

1.2 Brief Discussion of Nonclinical Findings

All pivotal nonclinical studies were conducted using oral administration of the drug, which is the clinical exposure route, and in accordance with US FDA GLP (21CFR58) as stated by the sponsor. Safety margins to expected human exposure were estimated using $C_{max,ss}$ = 687 nmol/L and $AUC_{0-24,ss}$ = 4740 nmol.h/L plasma exposure in T2DM subjects at the proposed maximum recommended human dose (MRHD) of 25 mg empagliflozin.

Pharmacology
Empagliflozin (BI 10773 or Jardiance™) is a selective inhibitor of sodium glucose co-transporter (SGLT) 2. SGLT2 is selectively expressed in the kidney S1 proximal tubule and is responsible for the majority (90%) of the renal reabsorption of glucose. Inhibition of SGLT2 by empagliflozin results in the excretion of glucose thereby producing glucosuria. In in vitro studies empagliflozin was a potent and selective inhibitor of human (h) SGLT2 relative to the closely related hSGLT1 with a selectivity of 4829-fold. In nonclinical models of diabetes, empagliflozin promoted glucose excretion, polyuria, increased sodium and chloride excretion, reduced urine osmolality and lowered plasma glucose in diabetic and non-diabetic animal models under conditions of hyperglycemia (oral glucose tolerance test).

Safety pharmacology assessment of cardiovascular, neurological, pulmonary, renal and gastrointestinal effects of empagliflozin did not identify significant liabilities.

Absorption, Distribution, Metabolism and Excretion
An oral dose of empagliflozin was rapidly absorbed and is approximately 94% and 89% bioavailable in mice and dogs, respectively, but only 31% bioavailable in the rat. In humans, absolute bioavailability was not measured. Consequently, the bioavailability can be inferred from a single oral dose trial in healthy volunteers where 50 mg of [^{14}C]empagliflozin was administered (Trial 1245.8). From this human trial, 75.5-77.4% of radioactivity in the plasma was the intact parent. From these results empagliflozin bioavailability resembles mice and dogs more than rats. Empagliflozin distributes rapidly to most rat tissues with low amounts distributing to brain, spinal cord, bone, bone marrow, eye, eye lens, testis and uveal tract. Similar volume of distribution (Vd) between rats (0.8 L/kg) and humans (1.05 L/kg) suggests that empagliflozin's tissue distribution profile in rats would be similar in human subjects.

In general, the metabolic disposition of empagliflozin was similar across nonclinical species and in humans. For example, following oral administration, intact empagliflozin was the predominant component in the plasma for mice (36-87%), rats (63-86%) and dogs (69-87%). In human volunteers, a single p.o. [^{14}C]empagliflozin at 50 mg, showed the majority of plasma empagliflozin is the unchanged parent (approx. 76%). Analysis

of the plasma in this human study showed the most abundant plasma metabolites were three empagliflozin glucuronides (M626/1 (CD00006135), M626/2 (CD00006134) and M626/3 (CD00006136)) representing 3.3-7.4% of the plasma radioactivity. In contrast, the major metabolite in the plasma of the mouse (10-65%), rat (6-29%) and dog (2-17%) is oxidative metabolite M482/1. Furthermore, in humans there are no major circulating metabolites at ≥10% and all human metabolites are found in the nonclinical species.

Empagliflozin has a longer half-life in humans (13 hours) than that in the mouse, rat, or dog (4-7 hours), suggestive of differential rates of renal elimination. As the concentration of empagliflozin in the plasma is qualitatively similar to that in the blood, blood clearance will approximate plasma clearance.

A difference in cross-species metabolism of potential relevance is that oxidative metabolism of empagliflozin predominates in rodents and dogs but is minor in humans. The oxidative pathway in all species involves production of an aldehyde intermediate which was identified as M466/2 in mice but was not identified in other species. The sponsor submitted data in October 2013 that the breakdown products of M466/2 are toxic to renal epithelial cells in vitro. The more stable breakdown products of M466/2 (e.g., M482/1, M380/1) are present in all species, including humans. Whether production of these metabolic products contributes to the renal toxicity observed in mice and dogs is uncertain; however, the risk to human subjects is considered low based on the minor degree of oxidative metabolism in humans versus rodents and dogs.

In vitro studies using microsomes or hepatocytes from nonclinical species and/or humans, showed empagliflozin was minimally metabolized and did not induce cytochrome P450 activity or greatly inhibit UGT activity. Efflux of empagliflozin was demonstrated via P-gp and BCRP transporters in vitro, and empagliflozin was also an in vitro substrate and inhibitor for multiple human transporters (OAT3, OATP1B1, OATP1B3 and OAT2B1 but not OAT1 and OCT2). However, these interactions occurred at micromolar concentrations and as the human C_{max} for empagliflozin is 0.687 μM at 25 mg (MRHD), drug-drug interactions are unlikely to be a clinical risk for empagliflozin.

Excretion of a single oral dose of [^{14}C]empagliflozin was detected as drug-related activity and was recovered predominantly in the feces of the nonclinical species (61 - 82%) followed by the urine (4 – 30%). Empagliflozin was the major component of the feces in the species tested, except in the dog. Similar results were obtained in human volunteers, where a single oral dose of [^{14}C]empagliflozin (50 mg), showed 54% and 41% of the dose was excreted in the urine and feces, respectively. Unchanged empagliflozin also represented 83% and 44% of the fecal and urinary radioactivity, respectively. Thus, nonclinical species are representative of human empagliflozin excretion process.

General Toxicology
Pivotal repeat dose studies were conducted in the Wistar (Han) rat and Beagle dogs up to 6 and 12 months duration, respectively. In the rat the empagliflozin exposure was 2-78x MRHD and in the dog the exposure was 17-261x MRHD.

Findings in the pivotal rat and dog studies were generally consistent with pharmacodynamic activity of empagliflozin, including dose-dependent increases in urinary glucose. The toxicological profile of empagliflozin supported the doses and duration of clinical studies conducted throughout drug development. At clinically relevant drug exposure pharmacodynamic action also resulted in reduced body weight (BW), increased food consumption (FC) and increased urinary volume.

In the 6 month pivotal rat study major target organs with toxicity included the kidney (cortical tubular dilatation, vacuolation and mineralization), adrenal gland (vacuolation) and the liver (vacuolation). A NOAEL was not established due to adrenal and hepatic vacuolation in all empagliflozin treatment groups with no vacuolation findings in the control animals. Adrenal vacuolation likely occurred due to osmotic and/or diuretic effect of enhanced glucose excretion (e.g. polyuria) and a resultant increase in aldosterone production due to enhanced sodium excretion as a result of SGLT2 inhibition, which is known for this drug class. The mechanism of hepatic vacuolation is unknown and was associated with a minimal (less than 2-fold) increase in hepatic transaminases. At the low dose, adrenal and hepatic vacuolation occurred at 2x and 5x MRHD in male and female rats, respectively, giving some margin to the clinical dose. Overall, the known pharmacodynamic activity of SGLT2 inhibitors for the adrenal gland, and the monitorable nature of human hepatic injury using liver function tests, lessens the clinical concern.

In the 52 week dog study a dose related increase in severity of vacuolation of the adrenal gland was also observed. Adrenal vacuolation likely occurred due to a similar mechanism as described above in the 6 month rat study above. Pharmacodynamic action also resulted in reduced body weight. Nephritis and cortical tubular degeneration with fibrosis was observed in the high dose animals, an effect that may not be related to pharmacodynamic activity but possibly to a nephrotoxic effect of empagliflozin's unidentified oxidative aldehyde metabolites. Overall target organ toxicity occurred at high exposure multiples (≥70x MRHD) and the safety margins to the final clinical dose are high, suggesting low clinical risk.

Reproductive Toxicology
Reproductive and developmental toxicity were assessed in fertility, early embryonic development and pre- and post-natal development animal studies. No effects were seen on mating and fertility indices in the females and the males at up to 155x MRHD. Systemically, empagliflozin reduced body weight gain in the males and females, resulting in a paternal and maternal NOAEL of 48x MRHD.

Empagliflozin was not teratogenic at 300 mg/kg (48x MRHD) in the rat. Higher exposure resulted in a skeletal malformation of bent limb bone at 154x MRHD. A

NOAEL for maternal toxicity was 22x MRHD due to reduced body weight, body weight gain and food consumption at ≥300 mg/kg. Empagliflozin was also not teratogenic at 300 mg/kg in the rabbit (128x MRHD). Higher exposure at 700 mg/kg (139x MRHD) resulted in abortions in 3 female rabbits that was likely due to reduced body weight gain during treatment. Due to the abortions and reduced body weight gain the NOAEL for maternal toxicity in rabbits was 300 mg/kg (128x MRHD).

A preliminary pre- and postnatal development study in the rat was terminated early (PND 19-24) due to lower body weight and 'small stature' of pups at a dose of 700mg/kg. The sponsor then conducted another pre- and postnatal study at lower doses of 0, 10, 30 and 100 mg/kg, a tissue distribution study in pregnant rats, and a rat lacteal excretion study. Lower doses of 1-16x MRHD in the second pre- and postnatal development study in the rat had no pathological effects in the dams up to the highest dose tested (100mg/kg). Lactational exposure during weaning led to reduced growth in the F_1 pups. The F_1 males also had a deficit in learning and memory at 16x MRHD at PND 22 but not at PND 62 resulting in a NOAEL of 4x MRHD. No effects were observed for the F_1 mating and reproductive performance (up to 16x MRHD) and there were no morphological changes in the F_2 pups (up to 16x MRHD).

The significant weight loss in pups in the pre- and postnatal developmental studies is clear evidence of drug exposure in the milk resulting in an adverse outcome. The reduced body weight/weight gain in the young pups may also explain the deficit in learning memory that did not occur in the older animals. The distribution of empagliflozin in fetal tissues and the rat milk (see below) constitutes a potential human clinical risk and necessitates discontinuation of nursing or exposure to empagliflozin in nursing mothers.

Empagliflozin was present at a low level in fetal tissues after a single oral dose to the dams at gestation day 18. In maternal milk the mean milk to plasma ratio ranged from 0.634 -5, and was greater than unity from 2 to 24 hours post-dose. The mean maximal milk to plasma ratio of 5 occurred at 8 hours post-dose, suggesting accumulation of empagliflozin in the milk. We note that this degree of distribution is likely a low estimate because the pregnant rats were administered only a single low oral dose rather than multiple higher doses of empagliflozin.

Standard reproductive toxicology studies with some other SGLT2 inhibitors have reported morphological effects in the kidneys (dilatation of renal tubules and pelvi) in juvenile rats and are considered secondary to the pharmacological action of SGLT2 inhibition. Due to differences in timing of kidney development/maturation between rats and humans, these adverse effects seen in the kidneys of juvenile rats are considered relevant to the assessment of reproductive and developmental risk. The substantial difference in the toxicity profile for effects on the kidney between the standard reproductive toxicology studies and juvenile animal studies may reflect exposure to the test-article during a 'critical window' of renal development. At the present time, juvenile toxicity studies in the rat for empagliflozin were not conducted by the sponsor. However, as empagliflozin was present in fetal tissues and in the maternal milk it

presents a potential developmental risk in the second/third trimesters of pregnancy and during nursing. The presence of an SGLT2 inhibitor in fetal tissues and/or in maternal milk of rats is considered sufficient evidence of potential human risk, which would be conveyed in drug labeling.

Genetic Toxicology
Empagliflozin was not mutagenic or clastogenic in an in vitro Ames assay, an in vitro mouse lymphoma L5178Y tk+/- assay, or in vivo assays: rat blood cell micronucleus assay and a rat bone marrow micronucleus assay. All metabolites of empagliflozin in human subjects have been indentified in mice and rats in vivo, and would have been evaluated for genotoxic potential in these studies. The weight of evidence suggests that empagliflozin and its identified metabolites are unlikely to be genotoxic in human subjects.

Carcinogenicity
Empagliflozin was assessed for its potential to induce tumors in two-year bioassays conducted in rats and mice. The two-year bioassays are intended to detect drug-induced tumors that arise from genotoxic as well as non-genotoxic mechanisms of action after approximately life-time exposure to an investigational drug.

In rats, empagliflozin did not increase the incidence of tumors in females at up to 72x MRHD. In male rats, empagliflozin dose-dependently increased the incidence of whole body/cavity hemangioma (due to the mesenteric lymph nodes) at 42x MRHD. Empagliflozin also numerically increased testicular Leydig cell tumors in males at 26x and 42x MRHD. This increase is likely to be related to drug treatment and is consistent with the results of several SGLT2 inhibitors. However, Leydig tumor formation in humans is rare and unlikely to be encountered in humans due to the known physiological differences between the rat and human for this tumor.

In mice, empagliflozin did not increase drug-related neoplasms in female mice at up to 62x MRHD. Renal tubular adenoma and carcinoma (combined) were significantly increased in males at 45x MRHD and was accompanied by a high incidence of renal atypical hyperplasia in the same group. Renal cystic tubular hyperplasia also increased at all doses in the empagliflozin-treated animals, particularly the males. The renal neoplasms also occurred in the presence of renal tubular injury (single cell necrosis, karyomegaly, hypertrophy, atrophy, cysts and pelvic dilatation) in the dosed groups. It is likely that the renal tumors were the culmination of chronic renal injury over the two-year dosing period. Causes of the chronic renal injury in mice are speculative, but likely causes include chronic renal compensation for higher filtration of electrolytes, glucose, and attendant fluid, and the sponsor argues that oxidative metabolite M466/2 is also contributive.

Overall, empagliflozin poses minimal carcinogenic risk to humans based on the high exposure multiples that caused tumor formation in a single sex in mice (45x MRHD) and rats (42x MRHD), respectively, and the high exposure multiples at the NOAEL in the rat (17-21x MRHD) and the mouse (4-7x MRHD).

The nonclinical carcinogencity studies in the rat and mouse did not identify any non-neoplastic or neoplastic findings in the lung and skin.

Special Toxicology Studies
Dermal and Eye Irritation Studies
Empagliflozin had no effect on dermal sensitization at concentrations up to 25% in a local lymph node assay and did not cause dermal irritation in rabbits. Empagliflozin at 10 mg was not irritating to the eye in rabbits.

1.3 Recommendations

1.3.1 Approvability

AP (Approval)
Pharmacology/Toxicology recommends approval of NDA 204629.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

Established Pharmacological Class (Highlights/Indications & Usage):
Empagliflozin will be described as a “sodium-glucose co-transporter 2 (SGLT2) inhibitor”. This is consistent with the EPC for the currently approved drug in the class, canagliflozin.

HIGHLIGHTS

----------USE IN SPECIFIC POPULATIONS----------
• Pregnancy: No adequate and well-controlled studies in pregnant women. Use during pregnancy only if the potential benefit justifies the potential risk to the fetus (8.1)
• Nursing mothers: Discontinue drug or nursing (8.3)

Section 8.1
Pregnancy Category C

Teratogenic Effects
Pregnancy Category C
There are no adequate and well-controlled studies of Jardiance™ in pregnant women. Based on results from rat studies and the mechanism of action of SGLT2 inhibitors, empagliflozin may affect renal development and maturation. In studies conducted in rats, empagliflozin crosses the placental barrier, reaches fetal tissues, and is present in lactational milk. During pregnancy, consider appropriate alternative therapies, especially during the second and third trimesters.

Jardiance™ should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

In a placental transfer and lacteal distribution study in pregnant and lactating rats, empagliflozin was present in fetal tissues and in lactational milk following a single oral 5mg/kg dose. The milk-to-plasma ratio of empagliflozin ranged from 0.6 to 5 and was greater than 1.0 from 2 to 24 hours post-dose.

Empagliflozin was not teratogenic in embryo-fetal development studies in rats and rabbits up to 300 mg/kg which approximates 48-times and 128-times, respectively, the maximum clinical dose of 25mg. At higher doses, malformations of limb bones increased in fetuses at 700 mg/kg or 154 times the 25 mg clinical dose in rats. In the rabbit, higher doses of empagliflozin resulted in maternal and fetal toxicity at 700 mg/kg, or 139 times the 25 mg clinical dose.

In pre- and postnatal development studies in pregnant rats, empagliflozin was administered from gestation day 6 through to lactation day 20 (weaning) at up to 100 mg/kg (~16 times the 25 mg clinical dose) without maternal toxicity. Significantly reduced body weight and a deficit in learning and memory (at post-partum day 22) were observed in the F_1 offspring at 100 mg/kg.

8.3 Nursing Mothers

It is not known if Jardiance™ is excreted in human milk. Empagliflozin is secreted in the milk of lactating rats reaching levels up to 5 times higher than that in maternal plasma. Since human kidney maturation occurs *in utero* and during the first 2 years of life when lactational exposure may occur, there may be risk to the developing human kidney due to inhibition of SGLT2 function in the renal tubules. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from Jardiance™, a decision should be made whether to discontinue nursing or to discontinue Jardiance™, taking into account the importance of the drug to the mother.

Section13.1

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

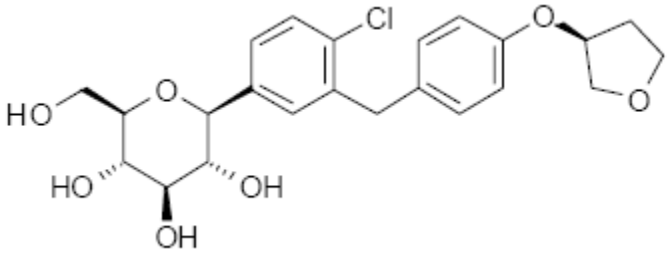
Carcinogenesis was evaluated in 2-year studies conducted in CD-1 mice and Wistar rats. Empagliflozin did not increase the incidence of tumors in female rats dosed at 100, 300 or 700 mg/kg (up to 72 times exposure from the maximum clinical dose of 25 mg). In male rats, hemangiomas of the mesenteric lymph node increased significantly at 700 mg/kg or approximately 42 times exposure from a 25 mg clinical dose. Empagliflozin did not increase the incidence of tumors in female mice dosed at 100, 300 or 1000 mg/kg/day (up to 62 times exposure from a 25 mg clinical dose). (b) (4)

Mutagenesis
Empagliflozin was not mutagenic or clastogenic with or without metabolic activation in the in vitro Ames bacterial mutagenicity assay, the in vivo L5178Y tk+/- mouse lymphoma cell assay, and an *in vivo* micronucleus assay in rats.

Impairment of Fertility
Empagliflozin had no effects on [redacted] (b) (4) up to the high dose of 700 mg/kg (approximately 155 times the 25 mg clinical dose in males and females, respectively).

2 Drug Information

2.1 Drug

CAS Registry Number	864070-44-0
Generic Name	Empagliflozin
Code Name	Jardiance™ / BI 10773 (BI 10773 XX)
Chemical Name	(1S)-1,5-anhydro-1-(4-chloro-3-{4-[(3S)-tetrahydrofuran-3-yloxy]benzyl}phenyl)-D-glucitol
Molecular Formula/Molecular Weight	$C_{23}H_{27}ClO_7$ / 450.91 g/mol
Structure or Biochemical Description	
Pharmacologic Class	Sodium glucose co-transporter 2 (SGLT2) inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 204629 was developed under IND 10215.

2.3 Drug Formulation

Empagliflozin will be marketed as a 25 mg film-coated tablet with the following composition:

Active ingredient: 25 mg of empagliflozin
Inactive ingredient: lactose monohydrate, microcrystalline cellulose, hydroxypropyl cellulose, croscarmellose sodium, colloidal silicon dioxide and magnesium stearate.

2.4 Comments on Novel Excipients

None.

2.5 Comments on Impurities/Degradants of Concern

In the drug substance impurities [redacted] (b) (4) were identified in the drug substance at [redacted] (b) (4) %. These impurities were qualified by their use in 4 and 13 week pivotal nonclinical studies in the rat and also by evaluation in an Ames assay and an in vitro micronucleus study in CHO cells which were negative.

2.6 Proposed Clinical Population and Dosing Regimen

Empagliflozin is indicated for treatment of Type 2 diabetes mellitus (T2DM). The recommended dose is 25 mg taken once daily.

2.7 Regulatory Background

NDA 204269 is the original submission of empagliflozin as a new molecular entity under 505(b)1

3 Studies Submitted

3.1 Studies Reviewed

Most studies were previously submitted and reviewed during the IND phase for empagliflozin. Summaries of nonclinical reviews from the IND are included in this NDA review. Nonclinical studies reviewed/summarized in this submission include:

Primary Pharmacology

BI 10773: In vitro Inhibition of SGLT2 and Selectivity vs SGLT1 and GLUT1 Transporters (Study# MD2006-007-Lab12, non-GLP, U06-1742).

Functional Inhibition of Human SGLT2 by BI 10773 (Empagliflozin) and its Metabolites BI1026317, BI 1026319 and BI1026318 (Study# MD2012-19-lab5, U12-2000-01)

Functional Inhibition of Human SGLT2 by BI 10773 (Empagliflozin) and its Metabolites BI 1026317, BI 1026319 and BI 1026318 (Study# md2012-19-lab5, U12-2000-01, non-GLP)

Functional Inhibition of Mouse SGLT1 and Mouse SGLT2 by BI 10773 (Study# MD2012-12-lab5, U12-1716-01, non-GLP)

Secondary Pharmacodynamics

Efficacy of the SGLT-2 Inhibitor BI 10773 XX After Single Oral Dosing in Diabetic Rodents and Normoglycemic Dogs (Study# MD2006_005_Lab8, U06-2214, non-GLP)

Effect of the SGLT2 Inhibitor BI 10773 on Glycaemic Control after Multiple Oral Dosing in Diabetic ZDF Rats (Study# MD2006/010/Lab8, U07-1071, non-GLP)

(b) (4) Pharmacology Data Report on Compound (b) (4), BI000010773 For Boehringer Ingelheim Pharma GmbH &Co.KG (Study# MD2005/012/Lab12, U06-1039)

(b) (4) Pharmacology Data Report on Compound (b) (4), BI000010773 For Boehringer Ingelheim Pharma GmbH &Co.KG (Study# MD2005/013/Lab12, U06-1040)

Safety Pharmacology

Neuropharmacological Profile (NPP) of BI 10773 XX in Rats (Study# 06R110, U07-3263, GLP)

Effects of BI 10773 XX on Behavior Assessed by Observation in a modified IRWIN-test and on Nocturnal Activity in Mice After Oral Administration of 3, 10 and 30 mg/kg (Study# GP2005/0350/0351/PH3, U05-2650, non-GLP)

Influence of BI 10773 XX on hERG-mediated potassium current in HEK293 cells and on Action Potential configuration in Isolated Guinea Pig Papillary Muscle (Study# GP2005/0378/0390/PHS, U05-2641, non-GLP)

Influence of BI 10773 XX (3, 10 and 30 mg/kg PO) on Hemodynamic and Electrocardiographic Parameters in Conscious Dogs (Study# GP2005/0396/PHS, U05-2639, non-GLP)

BI 10773 X: Evaluation of Cardiovascular (Hemodynamic) Function in Conscious Telemetered Beagles (Study# 06R117, U07-3536, GLP)

Effects of Oral Administration of BI 10773 XX in Doses of 3, 10 and 30 mg/kg on Vital Physiological Functions in Conscious Rats using a Telemetry / Plethysmography System (GP2005/0365/PH3, U05-2632, non-GLP)

Evaluation of Respiratory Function Following Oral Administration of BI 10773 XX in Rats (Study# 06R111, U07-3309, GLP)

Effects of BI 10773 XX (3, 10 and 30 mg/kg p.o.) on Renal and Liver Function in Conscious Rats (Study# GP2005/0398/0400/PH4, U06-1192, non-GLP)

Effects of BI 10773 XX (3, 10 and 30 mg/kg p.o.) on Gastric Emptying and Intestinal Transit in Rats (Study# GP2005/0379/PH4, U06-1108, non-GLP)

Effect of of BI 10773 XX (3, 10 and 30 mg/kg p.o.) on Gastric Secretion in Rats (Study# GP2005/0397/PH4, U06-1194, non-GLP)

PK/ADME

In vitro Evaluation of the Interaction of BI 10773 with Human P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP) (Study# DM-07-1066, U08-3676-01, non-GLP)

Quantitative Whole-Body Autoradiography of Rats Following Oral Administration of [14C] BI 10773 XX (Study# DM-08-1023, U08-3197-01, non-GLP)

In Vitro Evaluation of the Interaction of BI 10773 as an Inhibitor of Human Uptake Transporters OAT1, OAT3, OCT2, OATP1B1, OATP1B3, OATP2B1 and Human Efflux Transporters BCRP and MRP2 (Study# DM-11-1119, U12-3565-01, non-GLP)

In Vitro Evaluation of the Interaction of Empagliflozin with Human SLC Transporters using the Xenopus Oocyte System (Study# PK1230T, U12-1952-01, non-GLP)

In Vitro Assessment of Inactivation of Cytochrome P450 Isoforms 2C9, 2D6 and 3A4 by BI 10773 XX (Study# DM-08-1132, U09-3255, non-GLP)

In Vitro Assessment of Inhibition and Inactivation of Cytochrome P450s by BI 10773 XX and its Metabolites; CD00006134, CD00006135 and CD00006136 (Study# DM-10-1102, U10-3595-01, non-GLP)

In Vitro Evaluation of BI 10773 as an Inhibitor of Human Cytochrome P450: Determination of IC50 Values and Assessment of Drug Interaction Potential (Study# DM-06-1082, U07-3480, non-GLP)

In Vitro Metabolism of BI 10773 XX by Liver Microsomes and Hepatocytes of Multiple Species (Study# DM-08-1025, U09-3062-01, non-GLP)

In Vitro Assessment of the Induction Potential of BI 10773 in Primary Cultures of Human Hepatocytes (Study# DM-08-1026, non-GLP)

Preliminary Identification of BI 10773 Metabolites from Incubations with Hepatocytes of Rat, Dog and Human and in the Plasma of Mouse, Rat, Dog and Human (Study# DM-08-1104, U08-3899-01, non-GLP)

BI 10773 XX Metabolite Profiling and Tentative Metabolite Identification in the CD-1 Mouse (Study# DM-12-1028, U12-3547-01, non-GLP)

BI 10773 XX Metabolite Profiling and Tentative Metabolite Identification in the Han Wistar Rat (Study# DM-12-1027, U12-3548-01, non-GLP)

BI 10773 XX Metabolite Profiling and Tentative Metabolite Identification in the Beagle Dog (Study# DM-08-1098, U09-3023-01, non-GLP)

Interactions of BI 10773 XX with UGTs (Uridine Diphosphoglucuronosyltrasnferases) (Study# DM-11-1136, U12-3448-01)

Mass Balance in Male and Female CD-1 Mice After A Single Oral Dose of [^{14}C] BI 10773 XX (Study# DM-09-1105 (PK-09-09-1010), U09-3678-02, non-GLP)

Pharmacokinetics of BI 10773 XX After a Single IV or Oral Dose of BI 10773 XX to Male and Female CD-1 Mice (Study# DM-08-1091 (PK-08-1002), U08-3870-01, non-GLP)

^{14}C-BI 10773 XX Pharmacokinetic Study in CD-1 Mice (Study# DM-09-1003 (PK-08-1001), U10-3032-01, non-GLP)

Pharmacokinetic of [^{14}C]-BI 10773-derived Total Radioactivity in CD-1 Mice Administered a 1000 mg/kg Oral Dose of [^{14}C]-BI 10773 XX (BIPI Study No. PK-11-1028) (Study# DM-12-1040 (PK-11-1028), U12-3420-01, non-GLP)

Pharmacokinetics of BI 10773 in Han Wistar Rats (Study# DM-06-1020 (PK-06-1001), U06-3622, non-GLP)

Pharmacokinetics of BI 10773 XX, 14C-BI 10773XX and BI-10773 XX Total Metabolites in Male and Female Wistar Rats (Study# DM-06-1108 (PK-06-1015; PK-06-1016), U07-3499, non-GLP)

Pharmacokinetics of [^{14}C]-BI 10773-derived total radioactivity in Han Wistar rats Administered a 700 mg/kg oral dose of [^{14}C]-BI 10773 BIPI Study No. PK-11-1027 (Study# DM-12-1041, U12-3421-01, non-GLP)

Pharmacokinetics of BI 10773 XX after Intravenous (0.5 mg/kg) and Oral (5 mg/kg) Dosing in Beagle Dogs (Study# DM-06-1019 (PK-06-1002), U06-3413, non-GLP)

Pharmacokinetics and Mass Balance Study of [^{14}C]-BI 10773 XX in the Beagle Dog (BIPI Study PK-06-1017) (Study# U08-3654-01, non-GLP)

In Vitro Protein Binding of Bi 10773 XX: Rat, Dog, Mouse and Human Plasma, Human Serum Albumin and Human a1-Acid Glycoprotein (Study# DM-07-1001 (PK-06-1011), U07-3173, non-GLP)

In Vitro Protein Binding of BI 10773 XX in Rabbit Plasma (Study# DM-08-1100, (PK-08-1007), U09-3529-01, non-GLP)

In Vitro Blood Cell Partitioning of ^{1} C-BI 10773 XX in Wistar Rat, Dog and Human Blood (Study# DM-06-1083 (PK-06-1012) U06-3729)

^{14}C-BI 10773 XX Pharmacokinetic Study in CD-1 Mice (Study# DM-09-1003, U10-3032-01, non-GLP)

Pharmacokinetics of BI 10773 X, 14C-BI 10773 XX and BI 10773 XX Total Metabolites in Male and Female Wistar Rats (DM-06-1108, U07-3499)

General Toxicology

Acute Oral Toxicity Study in the Mouse with BI 10773 XX – Acute Toxic Class Method (Study# 06R109, U07-3238, GLP)

Acute Intraperitoneal Toxicity Study in the Mouse with BI 10773 XX – Acute Toxic Class Method (Study# 06R114, U07-3242, GLP)

Acute Oral Toxicity Study in the Rat with BI 10773 XX – Acute Toxic Class Method (Study# 06R108, U07-3234, GLP)

Acute Intraperitoneal Toxicity Study in the Rat with BI 10773 XX – Acute Toxic Class Method (Study# 06R114, U07-3242, GLP)

A 13-Week Oral Gavage Toxicity and Toxicokinetic Study in the CD-1 Mouse with BI 10773 XX (Study# 07R169, U09-3067-01)

2 Week Oral (Gastric Intubation) Range-Finding Toxicity Study in the Rat with BI 10773 (BIPI Study No. 05R214) (Study# U06-3381, non-GLP)

A 4-Week Subchronic Toxicity Study of BI 10773 XX Administered by the Oral (Gavage) Route to Wistar Rats with a 4-Week Recovery Period (Study# 06R073, U08-3183-01, GLP)

Thirteen-Week Oral (Gastric Intubation) Toxicity Study in the Rat with BI 10773 XX Followed by a Four-Week Recovery Period (Study# 07R036, U08-3744-01, GLP)

A 6-Month Oral (Gavage) Toxicity Study in Rats with a 3-Month Recovery Period (Study# 08R019, U09-3712-01, GLP)

2-Week Oral (Gastric Intubation) Range-Finding Toxicity Study in the Beagle Dog with BI 10773 (BIPI Study Number 05R215) (Study# U06-3404-01, non-GLP)

A 28-Day Toxicity Study of BI 10773 Administered by the Oral (Gavage) Route to Dogs with an 8-Week Recovery Period (Study# 06R010, U08-32010-01, GLP)

Thirteen-Week Oral (Gastric Intubation) Toxicity Study in the Beagle Dog with BI 10773 XX Followed by a Thirteen-Week Recovery Period (Study# 07R037, U08-3743-01, GLP)

A 26-Week Toxicity Study of BI 10773 XX Administered by Oral Gavage to Dogs with a 13-Week Recovery Period (Study# 08R026, U09-3679-01, GLP)

A 52-Week Toxicity Study of BI 10773 XX Administered by Oral Gavage to Dogs with a 13-Week Recovery Period (Study# 08R027, U10-3252-01, GLP)

DART

A Study of Fertility and Early Embryonic Development to Implantation of BI 10773 XX in Rats (Study# 08R008, U09-3133-01, GLP)

A Dose Range Finding Study of the Effects of BI 10773 XX on Embryo/Fetal Development in Rats (Study# 07R029, U08-3562-01, GLP)

A Study of the Effects of BI 10773 XX on Embryo/Fetal Development in Rats (Study# 07R030, U08-3556-01, GLP)

A Dose Range-Finding Study of the Effects of BI 10773 XX on Embryo/Fetal Developments in Rabbits (Study# 07R031, U08-3555-01, GLP)

A Study of the Effects of BI 10773 XX on Embryo/Fetal Developments in Rabbits (Study# 07R032, U08-3564-01, GLP)

Genetic Toxicology

BI 10773 XX: Mutagenicity Testing with L5178Y tk+/- Mouse Lymphoma Cells. Forward Mutation Assay (BI Study Number 06R088) (Study # 06R088, U07-3245)

L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with Three Treatment Conditions (Study # 06R233, U08-3198-01)

A 3 Day Micronucleus Assay in Rats Administered BI 10773 XX by Oral Gavage (Study No. 06R086) (Study# U07-3543, GLP)

A 3 Day Micronucleus Assay in Rats Administered BI 10773 XX by Oral Gavage (Study No. 06R141) (Study# U07-3233, GLP)

2-Week Oral (Gastric Intubation) Range-Finding Toxicity Study in the Rat with BI 10773 (BIPI Study Number 05R214) (Study# U06-3381, non-GLP)

(b) (4)

Carcinogenicity
104-Week Carcinogenicity and Toxicokinetic Study with BI 10773 in Mice (Study# 09R001, U12-3580-01, GLP)

104-Week Carcinogenicity and Toxicokinetic Study with BI 10773 in Mice (Study# 09R002, U12-3581-01, GLP)

3.2 Studies Not Reviewed

The following studies were submitted late in the NDA review cycle and were not reviewed. However, these studies were not needed to make a regulatory recommendation for empagliflozin. The submitted studies will be reviewed as a supplement to the NDA and if safety issues are identified, these will be addressed under the post-market requirement (PMR) regulations of FDASIA.

Quantitative Whole-Body Autoradiography in Male and Female Albino Mice After a Single Oral Administration of [14C]BI 10773 (Study# A073-12JS, U13-1808-01, non-GLP)

In Vitro Evaluation of the Uptake of Empagliflozin into Kidney Slices from Male and Female Mouse and Rat (Study# PK1301T, U13-1840-02, non-GLP)

Investigation on the In Vitro Metabolism of [14C]BI 10773 in Mouse, Rat and Human Kidney and Liver (Study# A337-13U, U13-1822-01, non-GLP)

BI 10773 XX Metabolite Profiling and Tentative Metabolite Identification in CD-1 Mouse Kidney (Study# DM-13-1002, U13-3477-02, non-GLP)

Bacterial Reverse Mutation Assay (Study# 13R096, U13-3656-01, non-GLP)

In Vitro Mammalian Cell Micronucleus Screening Assay in Chinese Hamster Ovary (CHO) Cells Under Three Treatment Conditions (Study# 13R097, U13-3655-01, non-GLP)

A 13 Week Renal Pathogenesis Study with BI 10773 in CD-1 Mice (Study# 12R139, U13-3467-01, non-GLP)

A 7 Day Renal Function and Toxicity Study with BI 10773 in CD-1 Mice (Study# 12R144, U13-3465-01, non-GLP)

A 7 Day Renal Function and Toxicity Study with BI 10773 in Wistar-Hanover Rats (Study# 12R145, U13-3466-01, non-GLP)

In Vitro Studies With Empagliflozin (BI 10773) in Mouse Primary Renal Tubular Epithelial Cells (Study# 13R083, U13-3468-01, non-GLP)

Structure-Toxicity-Relationship Assessment of BI 10773 M466 Metabolites (Study# 13R084, U13-3469-01, non-GLP)

(b) (4) In Vitro Pharmacology Screening Assays with Empagliflozin (BI 10773) and Comparator Compounds (Study# 13R085/ (b) (4) 100006632, U13-3470-01, non-GLP)

SelectScreen® Biochemical Kinase Screening Assay With Empagliflozin (BI 10773) and Comparator Compounds (Study# 13R086, U13-3471-01, non-GLP)

In Vitro Studies with BI 00737687 (M466/2) in Mouse Primary Renal Tubular Epithelial Cells (Study# 13R193, U13-3679-01, non-GLP)

Bioanalysis of M466/2 (BI00737687) and M380/1, and Identification of the (b) (4) from the degradation of M466/2 in Phosphate Buffer in the Presence of Glutathione (Study# DM-13-1129, U13-3897-01, non-GLP)

Mode-of Action and Relevance for Empagliflozin-Related Renal Tumors in the Mouse Carcinogenicity Study (Study# U13-3693-02, non-GLP)

Genotoxicity of BI 10773? In Particular Genotoxicity of Male Mouse Predominant Metabolite M466(2) (Study# U13-3894-01, non-GLP)

Bioanalysis of M466/2 (BI00737687) and M380/1 in Bacteria Reverse Mutation Assay Test Media Using Authentic Standard (Study# U13-3895-01)

3.3 Previous Reviews Referenced

None.

4 Pharmacology

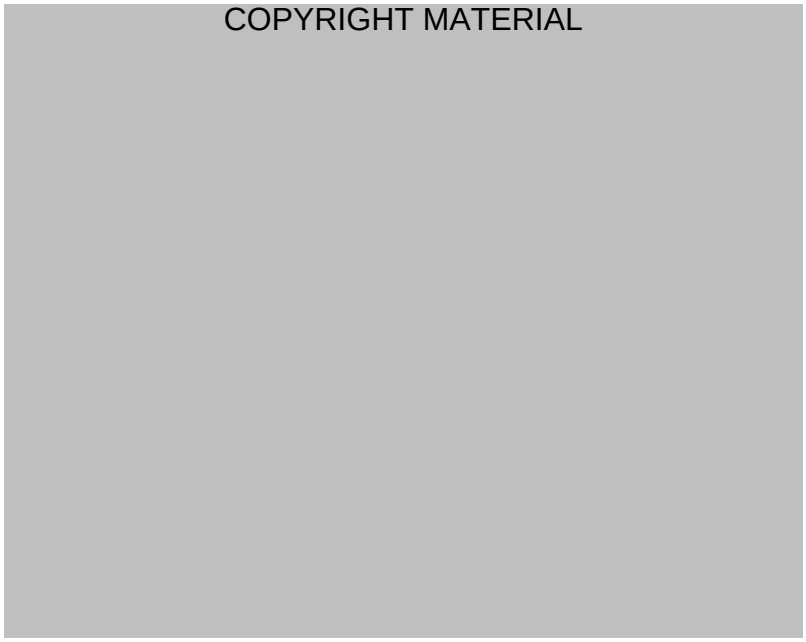
4.1 Primary Pharmacology

Empagliflozin is selective inhibitor of sodium glucose co-transporter (SGLT) 2 which is the major transporter involved in the reabsorption of glucose in the kidney.

Human embryonic kidney (HEK293) cells were stably transfected with cDNA for human (h) SGLT1 or hSGTL2, respectively, and used to measure the uptake of the glucose analogue ^{14}C-alpha-methyl-glucopyranoside (^{14}C-AMG). Empagliflozin was found to inhibit hSGLT1 or hSGLT2-mediated transport of ^{14}C-AMG with an IC_{50} of 6278 or 1.3 nmol/L, respectively, thus showing high potency and a 4829-fold selectivity for the inhibition of hSGLT2 (see Table 1 below).

Similarly, in Chinese Hamster Ovary (CHO) cells stably transfected with cDNA for rat (r) SGLT2, empagliflozin was also found to inhibit rSGLT2-mediated transport of ^{14}C-AMG with an IC_{50} of 1.7 nmol/L. In contrast, empagliflozin was found not to inhibit the glucose transporter GLUT1 which is found intrinsically in HEK293 cells. Empagliflozin at 10 μM was found to have 99% of the control (uninhibited) activity for the GLUT1-mediated transport of ^{14}C-desoxy-glucose (^{14}C-DOG) in HEK293 cells (see table 1 below). Low activity against the GLUT transporters is desired as these transporters play a critical role in glucose uptake in tissues such as the skeletal muscle and adipose tissue.

Table 1. Overview of In Vitro Pharmacology Effects of Empagliflozin in Nonclinical Pharmacology Studies



Grempler R., et., al., 2012 : Diabetes Obesity and Metabolism : 14(1) : 83-90, (2012)

Empagliflozin was not an appreciable inhibitor of mouse (m)SGLT1 with an IC_{50} of 28,000 nmol/L for the transport of ^{14}C-AMG in HEK293 cells. In contrast, empagliflozin inhibited mSGLT2-mediated transport of ^{14}C-AMG in HEK293 cells, with an IC_{50} of 1.7 nmol/L, thus showing 16,000-fold selectivity for mSGLT2 (see table above).

In similar studies, empagliflozin was found to have greater selectivity for in vitro inhibitory activity against hSGLT2 relative to hSGLT1 and the structurally related hSGLT4 (2500-3500-fold), hSGLT5 (for the uptake of mannose) (350-fold) and hSGLT6 (for the uptake of myo-inositol) (645-fold) (see table 1 above).

In another in vitro experiment using HEK293 cells, empagliflozin dose-dependently inhibited hSGLT2-mediated transport of ^{14}C-AMG with an IC_{50} of 3 nM, but the glucuronide metabolites of empagliflozin (BI 1026317, BI 1026319 or BI 1026318) were weak inhibitors of hSGLT2 with an IC_{50} of approximately 1-1.5 μM (see sponsor's table 2 below).

Table 2. IC_{50} For Inhibition of Human SGLT2 by BI 10773 and its Glucuronide Metabolites

A

BI 10773	pIC_{50}	Std. Error	IC_{50} (nM)
	8.52		
	8.39		
	7.99		
	8.88		
	8.70		
	8.51		
	8.64		
Average	8.52	0.11	3.0

B

BI 1026317	pIC_{50}	Std. Error	IC_{50} (nM)
	6.01		
	5.99		
	5.36		
	6.33		
	6.26		
	5.87		
	6.12		
Average	5.99	0.12	1019

C

BI 1026319	pIC_{50}	Std. Error	IC_{50} (nM)
	5.95		
	5.73		
	5.38		
	6.20		
	5.99		
	5.90		
	5.77		
Average	5.85	0.10	1426

D

BI 1026318	pIC_{50}	Std. Error	IC_{50} (nM)
	6.01		
	5.65		
	(~5.18)		
	6.05		
	5.78		
	6.00		
	6.01		
Average	5.92	0.07	1213

4.2 Secondary Pharmacology

Non-clinical efficacy of empagliflozin was assessed in db/db mice, Zucker diabetic fatty (ZDF) rats and Beagle dogs using secondary pharmacology/pharmacodynamic markers of urinary glucose excretion (glucosuria), polyuria and blood glucose lowering.

Single Dose Studies

Treatment of male db/db mice and male ZDF diabetic rats with a single dose of empagliflozin from 0.03 – 30 mg/kg resulted in a reduction of blood glucose in fed (prandial) animals (see sponsor's figures below).

Figure 1. Time Course of Blood Glucose in db/db Mice Following A Single Dose of Empagliflozin

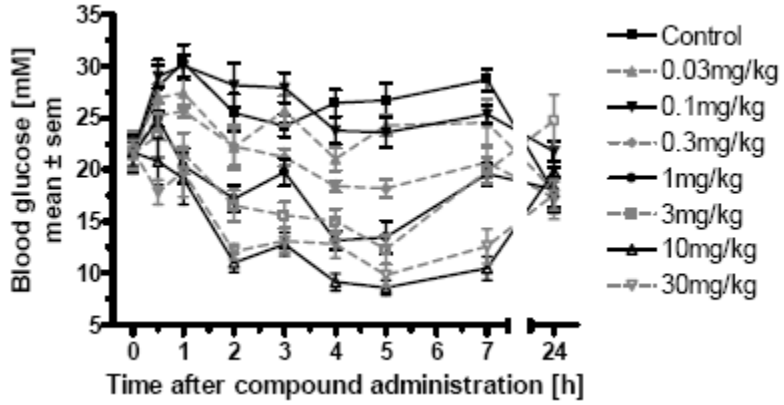
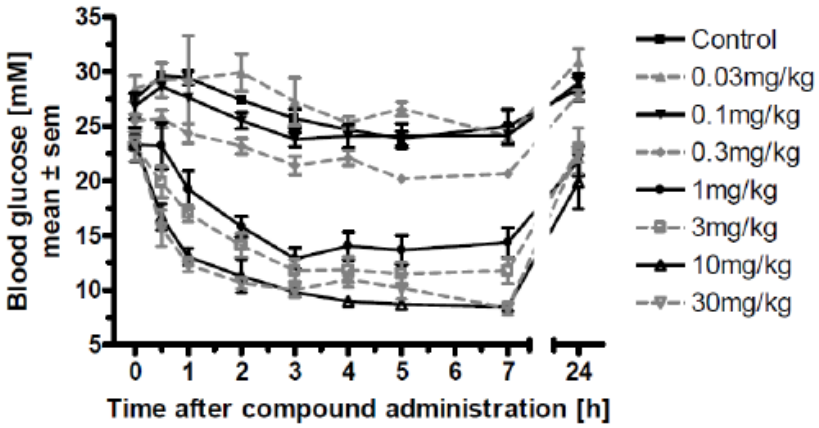


Figure 2. Time Course of Blood Glucose in ZDF Rats Following A Single Dose of Empagliflozin



In male ZDF rats and male db/db mice, a single oral exposure to empagliflozin at 3 mg/kg or 10 mg/kg in male normoglycemic dogs resulted in polyuria and glucosuria (see sponsor's table below)

Table 3. Urine Volume and Urinary Glucose in db/db Mice, ZDF Rats and Beagle dogs Following Empagliflozin at 3 or 10 mg/kg

	Vehicle		Empagliflozin	
Species	Volume [mL]	Glucose conc. [mM]	Urine volume [mL]	Glucose conc. [mM]
db/db mice	0.7 [a]	471.8[a]	2.1 [a]	660.8[a]
ZDF rats	8.8 ± 1.3 [a]	392.8 ± 137.5[a]	12.0 ± 1.8 [a] (p=0.21)	478.3 ± 33.3[a]
Beagle dogs	151.3 ± 71.7[b]	3.1 ± 0.6[b]	551.2 ± 98.4[b] (p=0.02)	310.3 ± 41.0[b] (p=0.02)

a mean of 0-3 h and 3-7 h after administration of vehicle or 3 mg empagliflozin/ kg b.w.
b 0-24 h after administration of vehicle or 10 mg/kg empagliflozin/ kg b.w.
Note: P values vs. control are given. Because the urine of db/db mice was collectively sampled, neither SEM nor p values are available. Please note that different sampling intervals and doses were used for rodents and Beagle dogs.

In male db/db mice and male ZDF rats exposed to empagliflozin between 0.1 - 30 mg/kg and subject to an oral glucose tolerance test (OGTT), empagliflozin dose-dependently reduced blood glucose at ≥ 0.3 mg/kg in each species and significantly reduced blood glucose 40-65% at ≥ 3 mg/kg in db/db mice and 81% at 30 mg/kg in ZDF rats, respectively, (see sponsor's figure below).

Figure 3. Blood Glucose AUC in db/db Mice Following An OGTT

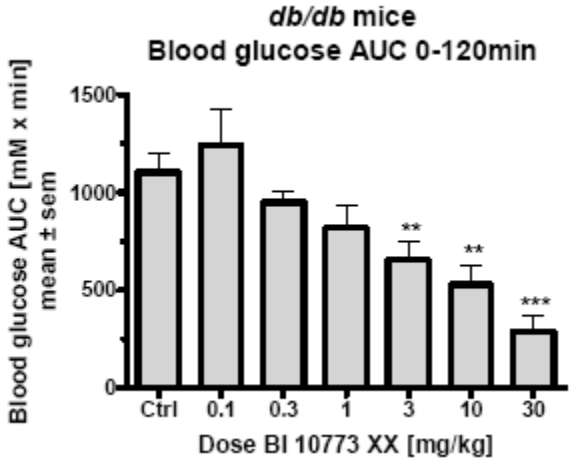
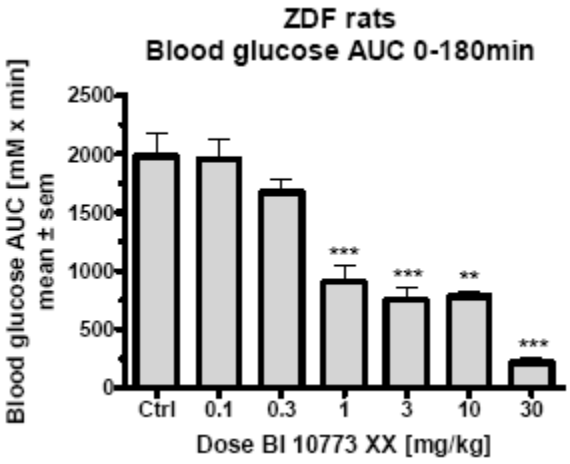


Figure 4. Blood Glucose AUC in ZDF Rats Following An OGTT



Multiple Dose Studies

Sub-chronic daily oral treatment with empagliflozin (0, 0.3, 1 or 3 mg/kg) in male diabetic ZDF rats for 5 weeks dose-dependently lowered the blood glucose 4-39%, relative to vehicle control, in fasted animals at day 37 post-dose (see sponsor's table below)

Table 4. Blood Glucose in ZDF Diabetic Rats Following Multiple Treatments with Empagliflozin

Baseline day -5 vehicle control	Day 37 vehicle control	0.3 mg/kg qd	1 mg/kg qd	3 mg/kg qd
7.4 ± 0.4 mM	15.2 ± 1.4 mM	14.6 ± 1.0 mM -3.9% (p = 0.7)	11.2 ± 1.0 mM -26.1% (p <0.05)	9.3 ± 0.6 mM -38.5% (p <0.01)

% difference between two means (empagliflozin dose vs vehicle-control after 5 week treatment)

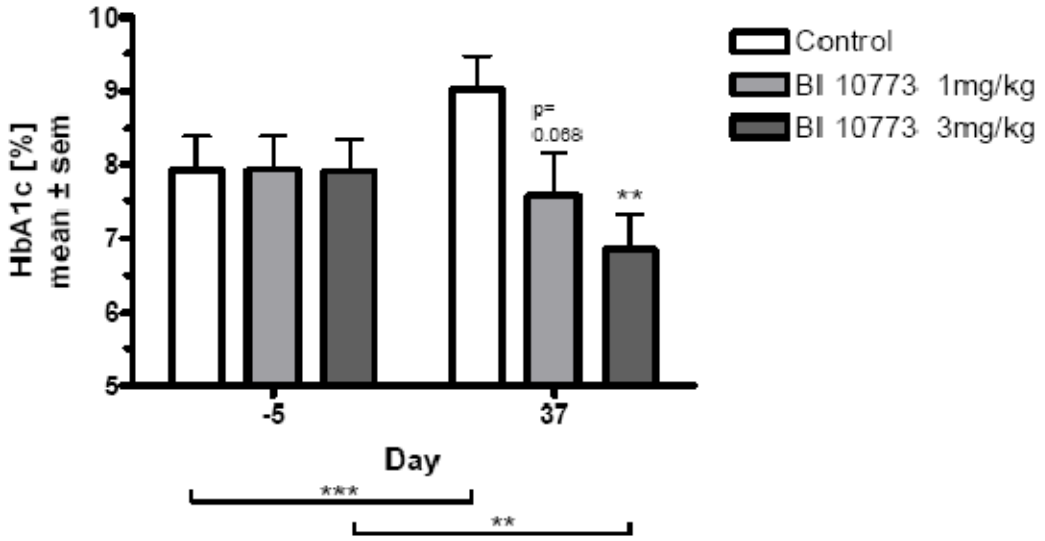
Blood glucose was also reduced 19% in fed male ZDF diabetic rats at day 23 post-dose with empagliflozin at 3 mg/kg (results not shown). Following an oral glucose tolerance test (OGTT), blood glucose $AUC_{0\text{-}3\ h}$ was also dose-dependently reduced 6-36% in male ZDF diabetic rats treated with empagliflozin from 0.3 – 3 mg/kg (see sponsor table below).

Table 5. Blood Glucose Exposure ZDF Diabetic Rats Following An OGTT

	% difference between two means of the glucose $AUC_{0\text{-}3h}$ (Empagliflozin dose vs. control)		
Species	0.3 mg/kg qd	1 mg/kg qd	3 mg/kg qd
ZDF rats	-6.0% (p = 0.3)	-31.3% (p <0.01)	-36.1% (p = 0.0001)

In control male animals HbA1c was 7.94 at baseline. At day 37 HbA1c had increased to 9.03, representing a significant (p = 0.0003) 14% increase. In the 3 mg/kg male ZDF diabetic rats, HbA1c had decreased from a baseline value of 7.93 to 6.84, representing a significant (p = 0.006) 14% reduction (see sponsor's figure below).

Figure 5. Baseline and Day 37 HbA1c values in Control and Empagliflozin (BI 10773) Treated ZDF Diabetic Rats



HbA1c values in ZDF rats before the start of treatment (Day -5) and after 5 weeks of multiple oral dosing of empagliflozin (Day 37). Indicated p-values are vs. control above bars and vs. baseline below figure (**p <0.01; ***p<0.001)

Empagliflozin was also screened in vitro for binding activity against a panel of unrelated receptors, ion channels, proteases, growth factors and transporters. Empagliflozin at 10 μM was found to have minimal (less than 30%) activity in this screening panel (see Appendix A)

4.3 Safety Pharmacology

Brief Summary
As part of the development program safety assessment for cardiovascular, neurological, pulmonary, gastrointestinal and renal effects of empagliflozin were conducted.

Neurological Effects
Male SD rats (n = 10/group) were treated with empagliflozin at 0, 500, 1000 or 2000 mg/kg per os (PO) and monitored for toxicological, neurological or body temperature effects at 15, 30 and 45 minutes post-dose and for up to 24 hr post-dose, respectively. Soft or wet feces was observed in all empagliflozin-treated rats, but CNS, or toxicological and body temperature effects were not observed.

Male SPF-mice(NMRI-Harlan) (n = 6/group) were treated with empagliflozin at 0, 3, 10 or 30 mg/kg PO and examined for general behavior and body temperature effects in a modified Irwin test over 24 hr, and also for nocturnal locomotor activity over 14 hr. No adverse CNS, body temperature or nocturnal locomotor activity effects were observed.

Overall, adverse CNS effects were not observed in the rat or mouse following treatment with empagliflozin at 0.5x-779x MRHD (on a body surface area basis).

Cardiovascular Effects
Empagliflozin had no effect on hERG potassium current in HEK293 cells, resulting in an IC_{50} of ≥30 μM. Empagliflozin also had no effect on action potentials generated from isolated guinea pig papillary muscles at up to 10 μM.

In an initial non-GLP study in conscious telemetered dogs (n = 2/sex/group), a single oral dose of empagliflozin at 3, 10 or 30 mg/kg administered in 24 hr increments, respectively, produced no changes in cardiovascular parameters. In a second GLP dog study in conscious telemetered dogs (n = 3sex/group), empagliflozin as a single oral treatment at 10, 30 or 100 mg/kg, with a 7 day washout period, respectively, also had no effect on heart rate, blood pressure, QTc interval or contractility. Empagliflozin as used in these studies was equivalent to 4x – 39x MRHD (on a body surface area basis)

The sponsor also evaluated empagliflozin in conscious telemetered male Wistar rats (n = 8/group, non-GLP). Empagliflozin as a single dose at 3, 10 or 30 mg/kg (0.5x – 12x MRHD on a body surface area basis) had no effect on systolic or diastolic blood pressure, heart rate or body temperature.

Overall, these results suggest minimal pro-arrhythmic potential for empagliflozin and a minimal impact on QT prolongation in humans.

Respiratory Effects
Empagliflozin was evaluated in conscious male Wistar rats (n = 8/group) placed in a plethysmography chamber. Empagliflozin as a single dose at 3, 10 or 30 mg/kg (0.5x – 12x MRHD on a body surface area basis) had no effect on respiration rate, tidal volume or body temperature.

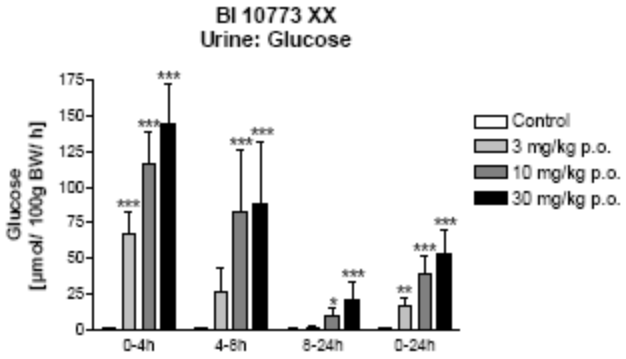
In another, plethysmography study, male Wistar (Han) rats (n = 4/group) were treated with empagliflozin at 0, 500, 1000 or 2000 mg/kg and placed in a plethysmographic chamber and evaluated for up to 6 hr post-dose. Empagliflozin up to 2000 mg/kg had no effect on respiratory rate, tidal volume or minute volume. Empagliflozin as used in this study is equivalent to 194x – 779x MRHD (on a body surface area basis).

Renal Effects

The impact of empagliflozin on renal and hepatic function was evaluated in Wistar rats following a single dose at 3, 10 or 30 mg/kg p.o. (20/sex/group) (equivalent to 0.5x – 12x MRHD on a body surface area basis). For the analysis male and female data were combined.

Urinary glucose excretion was dose-dependently and significantly increased at 0-4, 4-8 (10 and 30 mg/kg only), 8-24 (10 and 30 mg/kg only) and 0-24 hr post-dose (see sponsor's figure below).

Figure 6. Urinary Glucose Excretion Following a Single Oral Dose of Empagliflozin



Urinary sodium excretion was dose-dependently and significantly increased at 10 and 30 mg/kg from 0-4 hr post-dose and at 30 mg/kg at 0-24 hr post-dose, respectively (see sponsor's figures below). Urinary chloride was significantly increased at 30 mg/kg from 0-4 hr post-dose, but dose-dependently decreased and significant at 10 and 30 mg/kg at 4-8 hr post-dose, respectively (see sponsor's figure below)

Figure 7. Urinary Sodium Excretion Following a Single Oral Dose of Empagliflozin

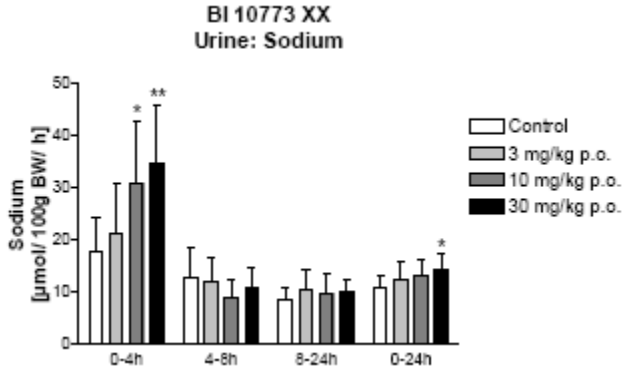
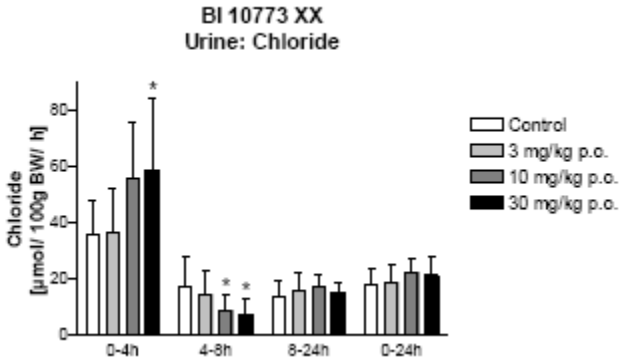
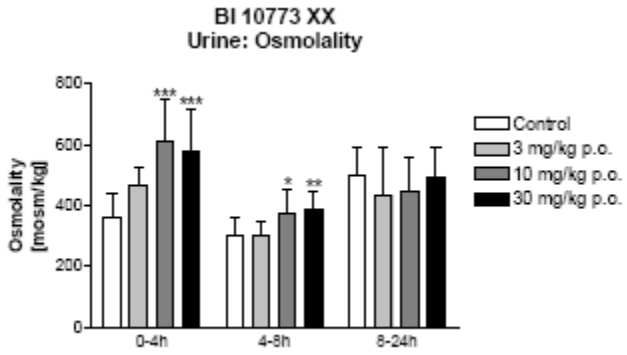


Figure 8. Urinary Chloride Excretion Following a Single Oral Dose of Empagliflozin



Urine osmolality was significantly increased at 10 and 30 mg/kg at 0-4 and 4-8 hr post-dose, respectively (see sponsor's figure below).

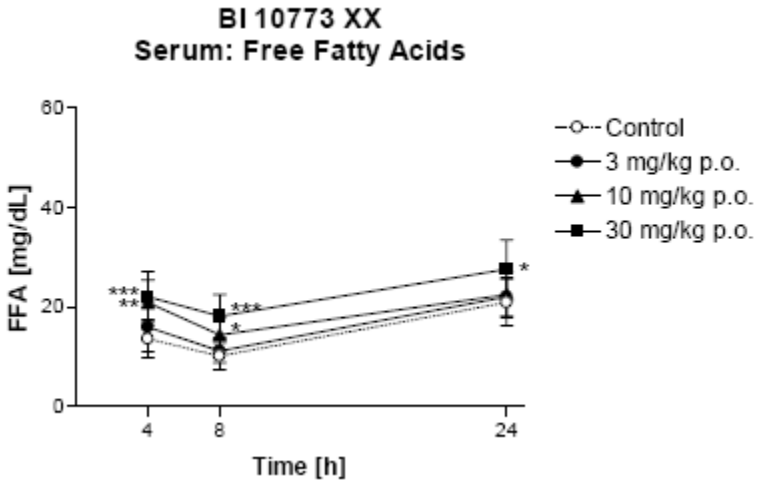
Figure 9. Urine Osmolality Following a Single Oral Dose of Empagliflozin



Empagliflozin as a single dose at up to 30 mg/kg (12x MRHD on a body surface area basis) had no impact on urine volume, potassium, calcium, magnesium, protein, albumin or urinary pH.

Empagliflozin dose-dependently and significantly increased serum free fatty acids (FFA) at 10 and 30 mg/kg, at 4 hr post-dose (see sponsor's figure below). FFA were also significantly increased at 10 and 30 mg/kg at 8 hr post-dose and at 30 mg/kg at 24 hr post-dose, respectively, but were within the historical control range for FFA. Increased FFA may be a compensatory mechanism for the urinary glucose loss observed.

Figure 10. Serum FFA Following a Single Oral Dose of Empagliflozin



Gastrointestinal Effects

To determine the effect of empagliflozin on gastric emptying, empagliflozin at 3, 10 or 30 mg/kg p.o., respectively was administered to fasted Wistar rats (5/sex/group), followed by the oral administration of a barium sulphate test meal. Empagliflozin had no effect on intestinal transit, however empagliflozin at 30 mg/kg increased gastric emptying by 33% (see sponsor's table below). Empagliflozin as used in this study is equivalent to 0.5x – 12x MRHD (on a body surface area basis). Although, this safety pharmacology study is an acute single dose study, GI effects of this drug class are known to occur in rodents. At high doses and multiple exposures, a pharmacodynamic effect of SGLT2 inhibitors results in increased calcium absorption and this is related to off-target inhibition of the closely related SGLT1 transporters in the GI tract. Additionally, this finding is usually associated with GI distension in rodents. Acute absorption of ionized calcium was also not evaluated in the present study and it is likely that multiple high doses of empagliflozin would be required to produce a GI effect, as was observed in the rodent carcinogenicity studies (midline swollen abdomen). The minimal increase in gastric emptying in the present study is unlikely to have a drug-drug interaction effect.

Table 6. Effect of Empagliflozin on Gastric Emptying and Intestinal Transit in the Wistar rat

BI 10773 XX	Control	3 mg/kg	10 mg/kg	30 mg/kg
Gastric emptying [mg/100g BW]	2123±481	1875±194	1956±309	1426±491 **
Diff. [%]		-12	-8	-33
Intestinal transit [%]	64±8	62±10	63±9	67±12
Diff. [%]		-3	-2	+4

In a second rat study 8 male Wistar rats were treated intraduodenally with empagliflozin at 3, 10 or 30 mg/kg (8/group) following surgical pylorus ligature. At 4 hr post-dose, empagliflozin had no effect on gastric pH, total acidity, volume or acid output (results not shown). The present study at 1x -12x MRHD (on a body surface area basis) shows empagliflozin to have a minimal effect on gastric acid secretion and thereby ulcerogenic activity.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Methods of Analysis
Plasma concentration of empagliflozin: LC/MS/MS assay with a lower limit of quantification of 50, 45, 50 and 100 ng/mL in the rat, mouse, rabbit and dog plasma, respectively.

Radioactivity measurement: Liquid scintillation counting of relevant samples and Quantitative whole-body autoradiography (QWBA). The detection limit for sample identification in plasma and blood were 50 and 75 dpm, respectively.

Metabolite identification: LC/MS/MS and authentic standards.

Pharmacokinetics
Pharmacokinetics following a single oral administration of empagliflozin at 250 mg/kg in CD-1 mice was characterized by a half-life (t½) of 4-6 h and exposure ($AUC_{0-\infty}$) that was slightly increased (1.3-fold) in female mice compared to male mice (see sponsor's table below). Intravenous administration of empagliflozin at 5 mg/kg in the CD-1 mice also produced a short a half-life (t½) of 0.6-1.6 h. The oral bioavailability of empagliflozin was high (90% males and 97% in females, respectively) in CD-1 mice following administration at 250 mg/kg (see sponsor's table below).

Table 7. PK of Empagliflozin Following Single Administration in CD-1 Mice

Pharmacokinetic parameter	Intravenous		Oral	
Dose (mg/kg)	5		250	
Gender	M	F	M	F
CL (mL/min/kg)	40.1	33.0	44.6 [a]	33.8 [a]
Vss (L/kg)	1.17	0.868	--- [b]	---
t½ (hr)	1.26	0.654	5.59	4.31
C_{max} (nM)	---	---	97,700	91,500
t_{max} (h)	---	---	0.67	0.33
$AUC_{0-\infty}$ (nM·hr)	4,610	5,600	207,000	273,000
MRT (hr)	0.488	0.433	2.52	4.05
Bioavailability (%)	---	---	89.8	96.7

[a] CL/F
[b] ---, not determined

A single oral administration of [^{14}C]-empagliflozin in CD-1 mice at 1000 mg/kg resulted in an approximately 2-fold higher exposure in the male mice compared to female mice. Of note, the half-life (t½) was 7.4 and 1.41 h in the male and female rats (see sponsor's table below). The gender difference in exposure was the reverse of CD-1 mice exposed to empagliflozin at 250 mg/kg described above.

Table 8. PK Parameters in CD-1 Mice with Empagliflozin at 1000 mg/kg

PK Parameter	Male	Female
C_{max} (nM)	356,000	260,000
t_{max} (h)	2	2
$AUC_{0-\infty}$ (nM·h) [a]	1,670,000	1,040,000
$t_{1/2}$ (h)	7.40	1.41
MRT (h)	6.24	3.45

[a] The percentage of AUC extrapolated was 5.5% for males and 4.9% for females.

Following a single intravenous administration (using 80% PEG: 20% saline vehicle) in male Wistar rats at 0.5 mg/kg the half-life was 3 hours (see sponsor's table below). With a single oral administration of empagliflozin at 5 mg/kg (using 100 % PEG 400 vehicle) in male Wistar rats the half-life was 6 hours and the bioavailability was 31% (see sponsor's table below).

Table 9. PK Parameters in Wistar Rats

BI 10773 Pharmacokinetic Parameters (Mean ± SD) in Male Han Wistar Rats		
Pharmacokinetic parameter	Intravenous	Oral
	Dosed in PEG 400:saline (80:20)	Dosed in 100% PEG 400
N	4	4
Dose (mg/kg)	0.5	5
CL (mL/min/kg)	14.8 ± 3.9	47.2 ± 7.4[a]
V_{ss} (L/kg)	0.818 ± 0.711	----
$t_{½}$[b](h)	3.64 ± 1.57	6.32 ± 2.29
C_{max} (ng/mL)	--	326 ± 54
t_{max} (h)	--	1 (1-2)[c]
$AUC_{0-\infty}$ (ng·h/mL)	595 ± 160	1,798 ± 293
Bioavailability (%)	--	31.0 ± 5.1

[a] CL/F
[b] Harmonic mean.
[c] Median and range.

Following a single oral administration of [^{14}C]-empagliflozin at 700 mg/kg in rats, the exposure ($AUC_{0-\infty}$) was 3-fold higher exposure in female rats compared to male rats. The half-life was 1.98 and 3.73 h in the male and female rats, respectively (see sponsor's table below).

Table 10. PK of BI 10773 Following A Single Oral or IV Administration in Rats

Parameter	Males					
	Rat 1	Rat 2	Rat 3	Rat 4	Mean	SD
C_{max} (nM)	40,200	62,200	48,900	110,000	65,200	30,900
t_{max} (h)	1	1	0.5	1	1[a]	(0.5-1)[a]
$AUC_{0-\infty}$[b] (nM·h)	132,000	188,000	204,000	350,000	219,000	92,800
$t_{1/2}$ (h)	1.59	1.77	3.08	1.49	1.98	0.739
MRT (h)	2.52	2.55	4.38	2.43	2.97	0.939
Parameter	Females					
	Rat 5	Rat 6	Rat 7	Rat 8	Mean	SD
C_{max} (nM)	184,000	114,000	87,300	120,000	126,000	40,900
t_{max} (h)	0.5	1	0.25	4	0.75[a]	(0.25-4)[a]
$AUC_{0-\infty}$[b] (nM·h)	618,000	487,000	681,000	1,240,000	758,000	334,000
$t_{1/2}$ (h)	1.26	1.42	7.00	5.24	3.73	2.85
MRT (h)	2.31	3.08	9.18	6.77	5.33	3.22

[a] Summary statistics for t_{max} are median (range).
[b] The mean percentage of AUC that was extrapolated was 21.0% in males and 5.0% in females.

Following intravenous (iv) infusion of [^{14}C]emapgliflozin at 0.5 mg/kg in the dog, the plasma half-life ($t_{1/2}$) was 22-31 h, which was also reflected in a low plasma clearance

rate. Following oral administration of [^{14}C]empagliflozin at 5 mg/kg, the plasma half-life ($t_{1/2}$) was 3.6 and 5.1 h in males and female dogs, respectively. Exposure via the intravenous (iv) or oral route of administration showed no gender differences. The oral bioavailability of BI 10773 was high (92-100 %) in dogs.

Table 11. PK of Empagliflozin Following A Single Oral or IV Administration in Dogs

Route	IV (0.5 mg/kg)		PO (5 mg/kg)	
Sex	M	F	M	F
CL (mL/min/kg)	1.76 ± 0.13	1.65 ± 0.03	1.73 ± 0.22 [a]	1.80 ± 0.13 [a]
Vss (L/kg)	0.836 ± 0.192	1.08 ± 0.56	--	--
$t_{½}$(hr)	22.0 ± 3.9	31.2 ± 13.3	3.60 ± 0.16	5.16 ± 1.88
C_{max} (nM)	--		16,100 ± 781	15,500 ± 1,500
t_{max} (hr)	--		1 ± 0	1 ± 0
AUC_{0-t} (nM·hr)	9,960 ± 720	10,500 ± 300	101,000 ± 12,500	96,300 ± 6,700
$AUC_{0-\infty}$ (nM·hr)	10,200 ± 700	10,800 ± 200	101,000 ± 12,600	96,800 ± 6,500
MRT (hr)	7.87 ± 1.43	10.9 ± 5.5	5.07 ± 0.17	5.54 ± 0.48
Absorption (%)	--	--	102	92.1

[a] CL/F

Absorption

The in vitro Caco-2 and (Mandin-Darby Canine Kidney) MDCK-MDR1 cell models were used to determine the rate of passage of empagliflozin. Caco-2 cells express both human p-gylcoprotein (P-gp) and the breast cancer resistance protein (BCRP) transporters and the MDCK-MDR1 cells express the stably transfected human P-gp transporter, respectively. Empagliflozin was found to be a substrate of both P-gp and BCRP, but did not inhibit P-gp or BCRP-mediated transport of probe substrates in these cell models. In MDCK-MDR1 cells empagliflozin primarily exhibited high secretory transport (basolateral (BL) to apical (AP) (BL- AP)), with only slight absorptive transport (AP - BL). Thus the BL- AP/AP – BL ratio was greater than 10 at all empagliflozin concentrations evaluated.

Absorption of empagliflozin following a single oral dose is rapid with C_{max} being achieved at (T_{max}) 0.33-2, 0.75-1 and 1 hr for the mouse, rat and dog, respectively. Following oral administration empagliflozin was eliminated with a half-life of 1.4-12, 1-6 and 3-6 hr for the mouse, rat and dog, respectively. Following IV administration empagliflozin was eliminated with a half-life of 0.6-0.9, 0.6-3.6 and 6.2-31 hr for the mouse, rat and dog, respectively. In humans empagliflozin at 0.5 to 800 mg in a single oral rising dose study in healthy volunteers, resulted in rapid absorption with a T_{max} of 1.5 – 2.5 hr and a half-life of 8.5 – 13.1 hr for doses of 2.5 to 800 mg (Study# 1245.1). In a 4- week multiple dose study in type 2 diabetes mellitus (T2DM) humans, empagliflozin at 10, 25 or 100 mg/day resulted in rapid absorption with a T_{max} of 1.5 – 1.8 hr and a half-life of 13.2 –16.5 hr (Study# 1245.4).

The oral bioavailability of empagliflozin is 31%, 94%and 89% in rats, mice and dogs, respectively.

Distribution

Tissue Distribution

Empagliflozin distributes to tissues rapidly following oral exposure in the rat. Concentrations of empagliflozin were slightly higher in the plasma compared to the blood but were qualitatively similar, implying blood clearance will approximate plasma clearance. The tissues with the highest drug concentrations were those involved with absorption and elimination processes such as the gastrointestinal tract, liver and kidney. All tissue with measurable concentrations of empagliflozin had maximum concentrations at 1 hour post-dose. Tissues where empagliflozin concentrations were absent included the brain, spinal cord, bone, bone marrow, eye, eye lens, testis and uveal tract. The empagliflozin concentration in all organs declined over 24 -168 hours indicating a lack of drug accumulation.

Stably transfected (human embryonic kidney) HEK-293 cells or *Xenopus laevis* oocytes expressing human (h) hOAT1, hOAT3, hOCT2, hOATP1B1, hOATP1B3, hOATP2B1 or BCRP or MRP2 transporters were used to determine the inhibitory/transport capacity of empagliflozin. Empagliflozin was found to be a substrate for some human distribution/excretion transporters (hOAT3, hOATP1B1 and hOATP1B3) but not all transporters (hOAT1 and hOCT2) (see sponsor's table below). Empagliflozin also inhibited hOAT3-, hOATP1B1-, hOATP1B3- and hOAT2B1-mediated transport of probe substrates, with an IC_{50} in the range 45-295 μM. Empagliflozin did not inhibit hOAT1- or hOCT2-mediated transport of probe substrates as the IC_{50} was greater than 1000 μM. Empagliflozin, did however, inhibit BCRP- and MRP2-mediated transport with an IC_{50} of 114 and 1399 μM, respectively (see sponsor's table below).

Table 12. Empagliflozin as a Substrate or Inhibitor of Various Transporters

Family	Transporter	Substrate	Inhibitor	IC_{50} (μM) [a]	I/IC_{50} [b]	I_2/IC_{50} [c]	DDI study recommended as potential inhibitor		Study number
							FDA [d]	EMA [e]	
ABC [f]	P-gp	Yes	No	>200	0.003	1.11	No	No	U08-3676
	BCRP	Yes	Yes	114	0.006	1.95	No	No	U08-3676, U12-3565
	MRP2	No	Yes	1399	-- [i]	--	--	--	U08-3676, U12-3565
SLC [g]	OATP1B1	Yes	Yes	71.8	0.010	--	No	No	U12-1952, U12-3565
	OATP1B3	Yes	Yes	58.6	0.012	--	No	No	U12-1952, U12-3565
	OATP2B1	ND [h]	Yes	45.2	0.015	--	--	--	U12-1952, U12-3565
	OAT1	No	No	>1000	0.0001	--	No	No	U12-1952, U12-3565
	OAT3	Yes	Yes	295	0.0004	--	No	No	U12-1952, U12-3565
	OCT2	No	No	>1000	0.0001	--	No	No	U12-1952, U12-3565

Plasma Protein Binding and Blood Partitioning

In vitro plasma protein binding of [^{14}C]empagliflozin to 4% human serum albumin (HSA) or to 0.07% α1-acid glycoprotein (AAG) was assessed using equilibrium dialysis for pooled mouse (CD-1), rat (Wistar), dog or human plasma. The mean plasma protein binding was similar in mouse (88%), rat (91%) and dog (89%) and slightly lower in human plasma (84%) (see sponsor's table below).

Table 13. In Vitro Plasma Protein Binding of [^{14}C]Empagliflozin in Various Species

^{14}C-Empagliflozin Protein Binding (% bound) [a]						
Conc. (µg/mL)	Mouse plasma	Rat plasma	Dog plasma	Human plasma	4% HSA	0.07% AAG
0.01	-- [b]	--	--	84.4 ± 0.6	83.6 ± 0.7	10.6 ± 2.8
0.1	88.0 ± 0.6	90.6 ± 0.3	88.2 ± 0.5	82.0 ± 0.4	80.3 ± 0.8	12.9 ± 6.5
0.3	--	--	--	84.5 ± 0.4	83.4 ± 0.7	14.1 ± 5.8
1	88.5 ± 0.3	90.1 ± 2.8	89.2 ± 0.3	84.1 ± 0.6	83.5 ± 0.2	16.6 ± 4.9
10	88.5 ± 0.2	91.2 ± 0.3	89.3 ± 0.3	--	--	--
40	87.5 ± 0.3	89.9 ± 0.2	88.2 ± 0.3	--	--	--

[a] Data are expressed as mean ± SD, n = 5.
[b] --, empagliflozin binding was not tested at this concentration.

In another study, in vitro plasma protein binding of [^{14}C]empagliflozin to pooled rabbit plasma was determined using equilibrium dialysis. The mean plasma protein binding in the rabbit was 91% and is similar to the mouse, rat and dog (see above).

The extent of blood cell partitioning was determined in the rat, dog and human with [^{14}C]empagliflozin at 0.3, 1 or 10 mg/Eq/mL. Partitioning into the blood cells was moderate with mean concentration in the blood cell (C_{bc}) to concentration in the plasma (C_p) ratios (C_{bc}/C_p) of 0.296, 0.253 and 0.301 in the rat, dog and human blood at 1 mg/Eq/mL , respectively. Increasing the [^{14}C]empagliflozin to 10 mg/Eq/mL minimally increased the partitioning in the rat and dog (partitioning at 10 mg/Eq/mL was not assessed in human blood) (see sponsor's table below).

Table 14. In Vitro Blood Cell Partitioning of [^{14}C]empagliflozin in Various Species

In Vitro Blood Cell Partitioning of ^{14}C-BI 10773 XX in Rat, Dog, and Human Blood		
Species	Concentration (µgEq/mL)	C_{bc}/C_p [a] (mean ± SD)
Rat	1	0.296 ± 0.057
	10	0.360 ± 0.065
Dog	1	0.253 ± 0.067
	10	0.243 ± 0.053
	50	0.343 ± 0.040
Human	0.3	0.323 ± 0.030
	1	0.301 ± 0.075

a Cbc/Cp is ratio of radioactivity concentration in blood cells to the concentration in plasma.

In CD-1 mice the ex-vivo measurement of blood cell partitioning following a single oral dose of 250 mg/kg [^{14}C]empagliflozin showed C_{bc}/C_p ratios in the range 0.250 – 0.280 with no gender or time differences (see sponsor's table below).

Table 15. Blood Cell Partitioning of [^{14}C]empagliflozin in CD-1 Mice Following A Single Oral Dose of [^{14}C]empagliflozin at 250 mg/kg

Gender	Time	C_{bc}/C_p [a]
Male	1 h	0.262 (10.4)
	6 h	0.250 (22.0)
Female	1 h	0.280 (7.18)
	6 h	0.266 (12.3)

[a] Only the blood samples from the first 4 mice for each time point were used for blood cell partitioning determination.

Overall, this shows no species differences in empagliflozin blood cell partitioning. In addition, concentrations of empagliflozin were higher in the plasma compared and to the blood, but were qualitatively similar, suggesting blood clearance will approximate plasma clearance.

Metabolism

Metabolic clearance of empagliflozin occurs to a minor extent in humans with the majority of the empagliflozin remaining unchanged (75%). Glucuronidation to three glucuronide metabolites (empagliflozin-2-O-, 3-O- and 6-O-glucuronide) are the most abundant metabolites in the human plasma (3-7%), with oxidative metabolism being a minor contributing pathway. By comparison, oxidative metabolism predominates in the nonclinical species with up to 31, 20 and 17% oxidative metabolism occurring in mice, rats and dogs, respectively.

In Vitro Metabolism

In vitro recombinant human CYP450 enzymes expressed in insect cells, show empagliflozin and its three glucuronide metabolites minimally inhibit CYP2C9, 2D6, 3A4, 2C8. In addition, empagliflozin and its three glucuronide metabolites minimally inhibit human liver microsomal CYP31A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4 metabolism of probe substrates with IC_{50}s in the 20 – 150 μM range (see sponsor's table below).

In vitro metabolism studies with empagliflozin at 0.1-10 μM and using rat, dog or human hepatocytes or microsomes, showed the in vitro half-life for metabolism of BI 10773 was 472, 1382 and >1579 minutes for each of the rat, dog and human hepatocytes, respectively. Empagliflozin was also found not induce human hepatocyte CYP1A2, 2B or 3A4 mRNA or enzyme activity against the CYP450 isozyme-specific substrates.

Further in vitro metabolism studies with empagliflozin at 10 μM in hepatocytes from the rat, dog and human showed four unique metabolites (M481-2, M625_2, M625_3 and M379). All metabolites from the nonclinical species were observed as metabolites in human plasma.

Table 16. IC_{50} Values for CYP450 Isoform Inhibition by Empagliflozin and its Glucuronide Metabolites

Compound	CYP450	Probe substrate	IC_{50} (μM)	Study number
Empagliflozin	1A2	Phenacetin	>100	U07-3480
	2B6	Bupropion	>50	U10-3595
	2C8	Amodiaquin	>100	U10-3595
	2C9	Diclofenac	~150	U07-3480
	2C19	(S)-Mephenytoin	>150	U07-3480
	2D6	Bufuralol	>150	U07-3480
	3A4	Midazolam	>150	U07-3480
	3A4	Testosterone	>150	U07-3480
CD00006134 (6-O-glucuronide)	2C8	Amodiaquin	>100	U10-3595
	3A4	Midazolam	>100	U10-3595
	3A4	Testosterone	>100	U10-3595
CD00006135 (2-O-glucuronide)	2C8	Amodiaquin	>20	U10-3595
	3A4	Midazolam	>100	U10-3595
	3A4	Testosterone	>100	U10-3595
CD00006136 (3-O-glucuronide)	2C8	Amodiaquin	>20	U10-3595
	3A4	Midazolam	>95	U10-3595
	3A4	Testosterone	>95	U10-3595

The potential for empagliflozin to inhibit UGT activity was evaluated in human liver microsomes. Empagliflozin inhibited UGT1A1-mediated metabolism of β-estradiol with an IC_{50} of greater than 50 μM and a Ki of 25 μM (see sponsor's table below). At steady state, the human C_{max} for empagliflozin is 0.687 μM at 25 mg, thus UGT1A1 is unlikely to be inhibited by empagliflozin in vivo.

Table 17. IC 50 for Empagliflozin-Mediated Inhibition of UGT1A1

UGT	IC_{50} (μM)[1]	K_i (μM)[2]
UGT1A1	>50	>25

1 Global fit, n=3

2 Competitive inhibition was assumed and K_i value was calculated as $IC_{50}/2$, since the concentration of substrate was equal to the apparent K_m value.

The recombinant UGT isoforms responsible for the formation of empagliflozin glucuronides were UGT1A3, 1A8, 1A9 and 2B7 (see sponsor's table below).

Table 18. UGT Isoforms involved in Empagliflozin Metabolism

Metabolite	BI 10773 XX (10 µM)	BI 10773 XX (687 nM)
CD00006134 (BI 10773-6-O-glucuronide)	BLOQ[1]	BLOQ
CD00006135 (BI 10773-2-O-glucuronide)	rUGT2B7	BLOQ
CD00006136 (BI 10773-3-O-glucuronide)	rUGT1A3, rUGT1A8, rUGT1A9, rUGT2B7	rUGT1A3, rUGT1A8, rUGT1A9, rUGT2B7

1 BLOQ: below limit of quantitation:
Limit of quantitation for BI 10773 (10 µM): 4.57 nM
Limit of quantitation for BI 10773 (687 nM): 0.823 nM

In Vivo Metabolism

Ten metabolites were identified in the plasma from in vivo 13-week toxicology studies conducted in mice, rats, dogs and from single dose and multiple dose studies in humans, including the four metabolites that were identified in the in vitro studies (see *in vitro* section above). This suggests in vivo and in vitro metabolism differences with a more complex metabolism process occurring in vivo (see sponsor's figure below). All human metabolites were identified in the non-clinical species with the exception of metabolite M625_4, which is a glucuronide product detected in trace amounts in concentrated human plasma. The 10 metabolites consisted of oxidation and glucuronide products of empagliflozin (see sponsor's figure below).

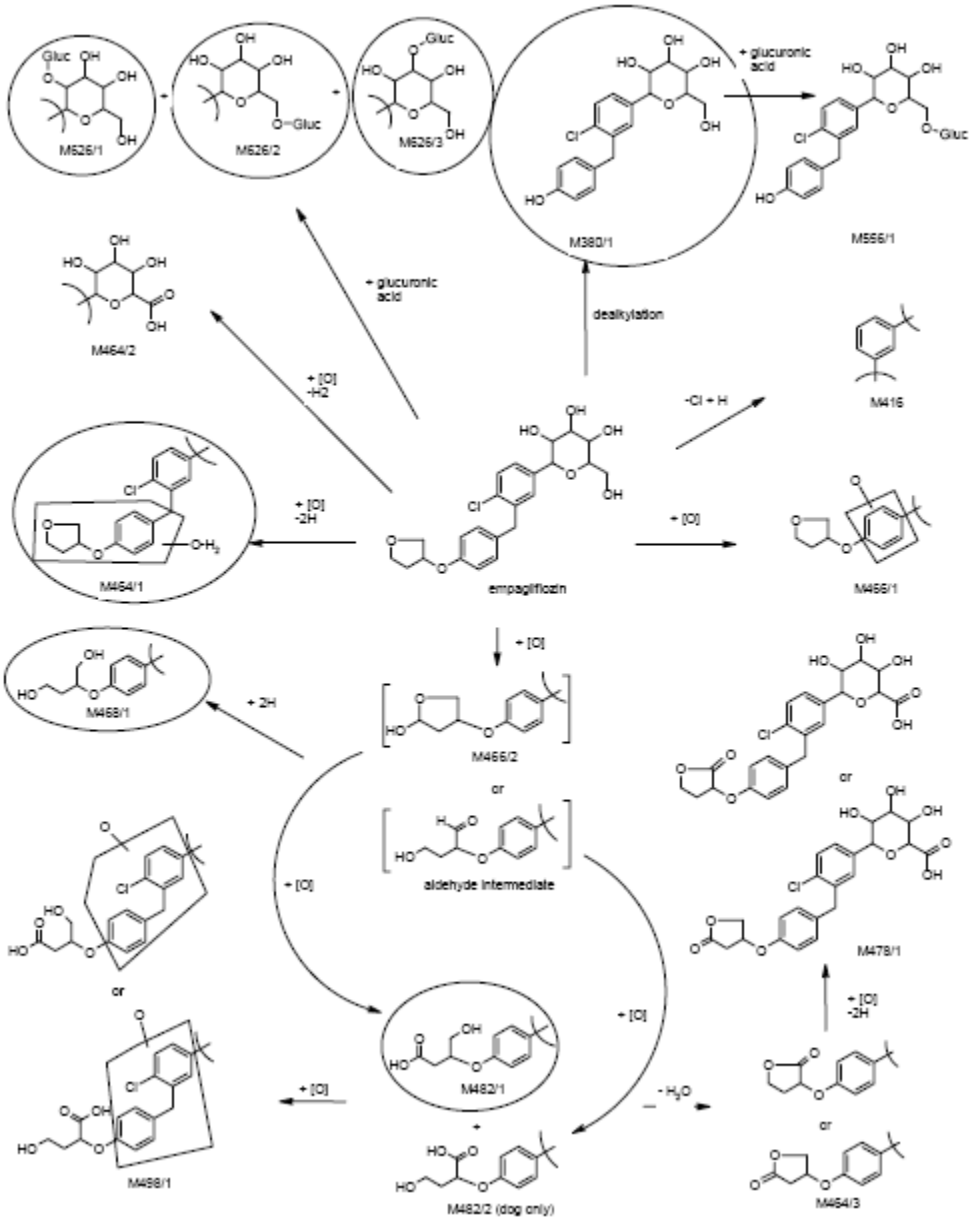
As a percentage of the plasma profile empagliflozin was the predominant component in the plasma of the mouse (36-87%), rat (63-86%), dog (67-89%) and humans (76%). In vivo metabolite profiles were qualitatively similar in all species tested. However, in humans glucuronidation (metabolites M626/1, M626/2 and M626/3) was the major metabolic pathway with oxidative metabolism occurring to a minor extent (see sponsor's table below) and is consistent with minimal cytochrome P450 activity observed in vitro. In contrast, oxidation was the major metabolic pathway in the nonclinical species.

Table 19. Empagliflozin Metabolite Profile in Human Plasma, Feces and Urine Following 50 mg p.o.

Compounds	Human Male Plasma						Feces + Urine (95.6% of dose) [a]				
	2 hr		6 hr		12 hr		Feces (41.1% dose) [a]		Urine (54.5% dose) [a]		Total
	% [^{14}C] [b]	nM	% [^{14}C] [b]	nM	% [^{14}C] [b]	nM	% [^{14}C] [b]	% dose [a]	% [^{14}C] [b]	% dose [a]	% dose [a]
M482/1	1.2	24.3	1.8	17.1	3.1	11.5	4.6	1.9	5.2	2.8	4.7
M626/1[c]	6.2	109	5.0	42.2	5.2	19.3	--[f]	--	14.4	7.8	7.8
M626/2[d]	3.7	62.8	6.0	49.4	3.3	12.3	--	--	3.9	2.1	2.1
M468/1	0.4	5.6	0.2	1.5	--	--	1.4	0.6	--	--	0.6
M626/3[e]	7.4	127	6.3	53.4	5.4	20.1	--	--	24.1	13.2	13.2
M464/1	0.5	8.5	0.4	3.1	1.1	4.1	2.6	1.1	1.5	0.9	2.0
Empagliflozin	77.4	1320	75.5	638	76.2	283	82.9	34.2	43.5	23.7	57.9
Total	96.8	1660	95.0	805	94.3	351	91.5	37.8	92.6	50.5	88.3

[a] % of dosed radioactivity.
[b] % of sample radioactivity.
[c] Authentic standard CD00006135 for M626/1 was available.
[d] Authentic standard CD00006134 for M626/2 was available.
[e] Authentic standard CD00006136 for M626/3 was available.
[f] Not detected.

Figure 11. Empagliflozin and Its Metabolites Identified in Multispecies Hepatocytes and Plasma Samples (Human Metabolites are circled)



Mice

Following a single oral dose of [^{14}C]empagliflozin at 1000 mg/kg, empagliflozin was the predominant component in the plasma of the mouse (36-87%) see sponsor's tables below). Oxidative metabolite M482/1 was the most abundant metabolite in the mouse plasma at 20-31% and 10-31% of the radioactivity in males and females, respectively (see sponsor's tables below and figure 11 above for the structure). Oxidative metabolites M464/1 was the next most abundant plasma metabolites at >10% in the male and female mice. The remaining metabolites were all present at <10%.

Table 20. Empagliflozin Metabolite Profile in Mouse Plasma, Feces and Urine - Male

Compounds	Male mouse plasma						Male mouse feces + urine				
	1h		4h		8h		Feces (81.7% dose)[a]		Urine (15.4% dose)[a]		Total
	% [^{14}C][b]	nM[c]	% [^{14}C][b]	nM[c]	% [^{14}C][b]	nM[c]	% [^{14}C][b]	% dose[a]	% [^{14}C][b]	% dose[a]	% dose[a]
M556/1	2.3	7260	3.3	4180	2.7	824	-	-	-	-	-
M482/2	-[e]	-	-	-	5.2	1580	-	-	-	-	-
M482/1	19.2	59800	24.3	31200	31.1	9540	16.9	13.8	51.7	8.0	21.8
M468/1+ M380/1[d]	2.2	6970	6.3	8070	4.5	1370	3.3	2.7	35.4	5.4	8.1
M464/2	-	-	-	-	-	-	-	-	0.7	0.1	0.1
M626/3[d]	6.0	18600	2.6	3340	3.7	1130	-	-	9.5	1.5	1.5
M464/1	6.0	18900	12.0	15300	14.6	4480	1.2	1.0			
BI 10773 XX	63.2	197000	51.5	66000	36.4	11200	77.8	63.6	2.2	0.3	63.9
Total	98.9	309000	100	128000	98.2	30100	99.2	81.1	99.4	15.3	95.4

[a] % of dosed radioactivity[1].
[b] % of sample radioactivity.
[c] Expressed in nM.
[d] Authentic standard was available.
[e] Not detected.

Table 21. Empagliflozin Metabolite Profile in Mouse Plasma, Feces and Urine - Female

Compounds	Female mouse plasma						Female mouse feces + urine				
	1h		4h		8h		Feces (79.4% dose)[a]		Urine (19.9% dose)[a]		Total
	% [^{14}C][b]	nM[c]	% [^{14}C][b]	nM[c]	% [^{14}C][b]	nM[c]	% [^{14}C][b]	% dose[a]	% [^{14}C][b]	% dose[a]	% dose[a]
M556/1	0.5	1580	-[e]	-	3.1	729	-	-	1.4	0.3	0.3
M482/2	-	-	-	-	-	-	-	-	-	-	-
M482/1	9.5	28400	17.7	21300	30.5	7100	11.3	9.0	50.7	10.1	19.0
M468/1+ M380/1[d]	1.1	3380	1.9	2310	2.8	657	2.3	1.8	15.2	3.0	4.9
M464/2	-	-	-	-	-	-	-	-	-	-	-
M626/3[d]	-	-	-	-	1.2	273	-	-	6.3	1.3	1.3
M464/1	0.9	2700	22.8	27500	13.8	3210	1.1	0.9			
BI 10773 XX	87.3	262000	57.7	69500	47.9	11100	85.3	67.7	26.4	5.3	73.0
Total	99	298000	100	121000	99.4	23100	100	79.4	100	19.9	98.4

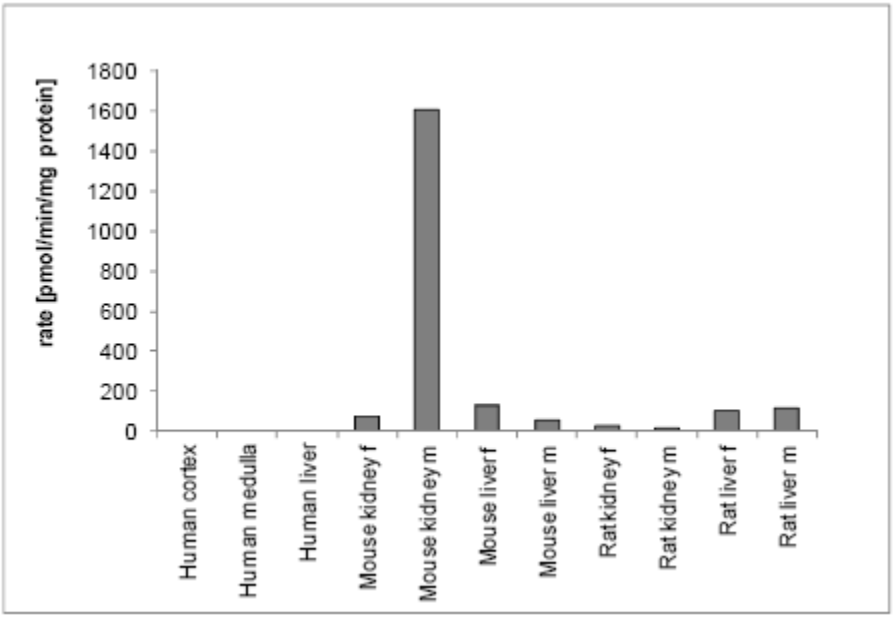
[a] % of dosed radioactivity[1].
[b] % of sample radioactivity.
[c] Expressed in nM.
[d] Authentic standard was available.
[e] Not detected.

In follow-up *in vitro* mechanistic studies, the sponsor incubated empagliflozin with kidney and liver microsomes in the mouse (8.9-10 μM), rat (10 μM) and humans (9.3 μM) and determined metabolite formation via LC-MS and metabolite identification using NMR. Using this method the sponsor identified a new oxidative metabolite M466/2 and an unidentified aldehyde intermediate that occurred predominantly in the male mouse kidney, to a much lesser extent in the female mouse kidney, mouse liver, rat kidney, rat liver but not in human kidneys or human liver following exposure to empagliflozin (see sponsor's table below and figure 12 below).

Table 22. In Vitro Empagliflozin Metabolites in Mouse, Rat or Human Kidney or Liver Microsomes

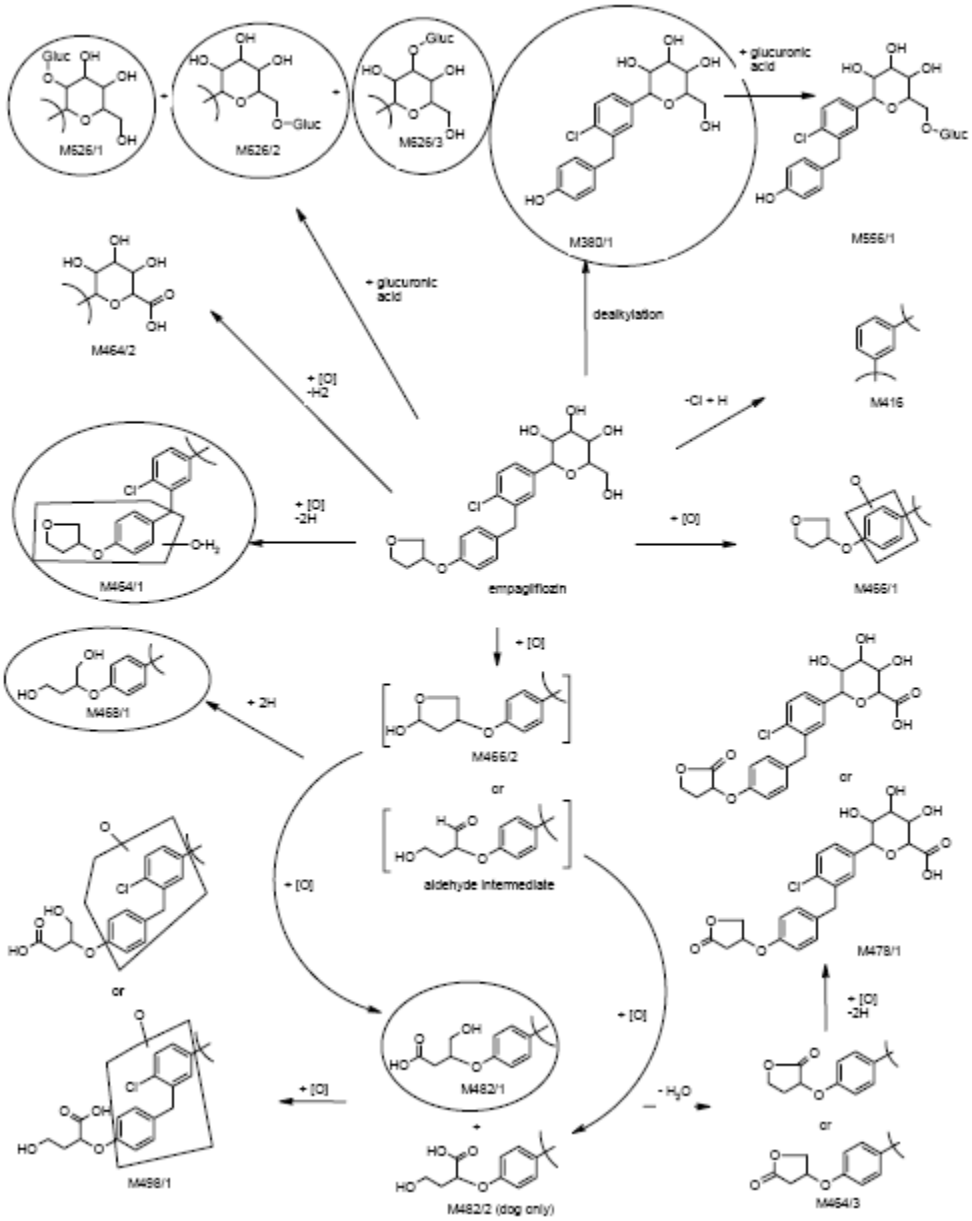
			metabolites identified by LC/MS				
Species	Tissue	Sex	M482(1)	M468(1)	M380(1) EX609	M466(2)	M464(1)
Human	Kidney, cortex	1m/2f	-	-	-	-	-
Human	Kidney, medulla	1m/2f	-	-	-	-	-
Human	Liver	6m/6f	-	-	-	-	-
Mouse	Kidney	10f	-	-	-	+	-
Mouse	Kidney	10m	-	-	+	+	+
Mouse	Liver	10f	-	-	-	+	-
Mouse	Liver	10m	-	-	-	+	-
Rat	Kidney	2f	-	-	-	+	-
Rat	Kidney	3m	-	-	-	+	-
Rat	Liver	2f	-	-	-	+	-
Rat	Liver	3m	-	-	-	+	-

Figure 12. Formation of Metabolite M466/2 in the Liver and Kidneys of the Mice, Rats and Humans



This finding is consistent with the known differences of empagliflozin metabolism in humans (glucuronidation) compared to that in mice (oxidation) (see sponsor's figure below). In the mouse carcinogenicity study, renal tumor formation was associated with degenerative tubular changes and this possibly could be due to the aldehyde intermediate metabolite. In addition, the sponsor proposes metabolite M466/2 and its breakdown products as key components in the mechanism of tumor formation in male mice at high doses. Due to the late submission of the mechanistic data to the NDA in the review cycle, the plausibility of the sponsor's proposal will be determined in a supplemental review to the NDA.

Figure 13. Human and Mouse Metabolism Pathways (Human Metabolites are circled)



Rats

Following a single oral dose of [^{14}C]empagliflozin at 700 mg/kg, empagliflozin was the predominant component in the plasma of the rat (63-86%). Oxidative metabolite M482/1 was the most abundant metabolite in the rat plasma at 18-20% and 6-10% of the radioactivity in male and female rats, respectively (see sponsor's tables below). Co-eluting metabolites M464/1 and M626/3 were the next most abundant plasma metabolites at >10% in the male rats. The remaining metabolites were all present at <10%.

Table 23. Empagliflozin Metabolite Profile in Rat Plasma, Feces and Urine - Male

Radioactivity contributions of [^{14}C]-BI 10773 XX and metabolites in male rats									
Compounds	Male rat plasma				Male rat feces + urine				Total
	0.5h		4h		Feces (79.7% dose)[a]		Urine (3.5% dose)[a]		
	% [^{14}C][b]	nM[c]	% [^{14}C][b]	nM[c]	% [^{14}C][b]	% dose[a]	% [^{14}C][b]	% dose[a]	% dose[a]
M482/1	20.4	14600	17.7	6840	9.7	7.7	38.2	1.3	9.1
M468/1+ M380/1[D]	3.8	2710	3.7	1430	2.9	2.3	15.9	0.6	2.9
M626/3[D]	10.9	7800	14.0	5410	-[e]	-	12.8	0.4	0.4
M464/1					-	-			
BI 10773 XX	62.8	44900	64.6	25000	86.4	68.9	33.1	1.2	70.0
Total	97.9	70000	100.0	38700	99.1	79.0	100	3.5	82.5

[a] % of dosed radioactivity[1].
[b] % of sample radioactivity.
[c] Expressed in nM.
[d] Authentic standard was available.
[e] Not detected.

Table 24. Empagliflozin Metabolite Profile in Rat Plasma, Feces and Urine - Female

Radioactivity contributions of [^{14}C]-BI 10773 XX and metabolites in female rats									
Compounds	Female rat plasma				Female rat feces + urine				
	0.5h		4h		Feces (69.4% dose)[a]		Urine (7.2% dose)[a]		Total
	% [^{14}C][b]	nM[c]	% [^{14}C][b]	nM[c]	% [^{14}C][b]	% dose[a]	% [^{14}C][b]	% dose[a]	% dose[a]
M482/1	6.3	7590	9.8	8370	16.0	11.1	18.9	1.4	12.4
M468/1+ M380/1[D]	1.4	1700	2.6	2220	4.5	3.1	13.3	1.0	4.1
M626/3[D]	3.1	3690	-[e]	-	-	-	15.1	1.1	2.8
M464/1			-	-	2.5	1.7			
BI 10773 XX	80.0	96100	86.0	73400	70.0	48.6	50.9	3.7	52.3
Total	90.8	109000	98.4	84000	92.9	64.5	98.2	7.1	71.6

[a] % of dosed radioactivity[1]
[b] % of sample radioactivity.
[c] Expressed in nM.
[d] Authentic standard was available.
[e] Not detected.

Dogs

Following a single oral dose of [^{14}C]empagliflozin at 5 mg/kg, empagliflozin was the predominant component in the plasma of the dog (64-89%). Oxidative metabolite M482/1 was the most abundant metabolite in the dog plasma at 2-17% and 3-10% of the radioactivity in male and female dogs, respectively (see sponsor's tables below). The remaining metabolites were all present at <10%.

Table 25. Empagliflozin Metabolite Profile in Dog, Plasma, Feces and Urine - Male

Oral dose of 5 mg/kg [^{14}C]empagliflozin in male beagle dog												
Compounds	RT	Dog Plasma						Feces + Urine (91.0% of dose) [a]				
		1 hr		4 hr		8 hr		Feces (61.4% dose)		Urine (29.6% dose)		Total
		%[^{14}C] [b]	nM	%[^{14}C]	nM	%[^{14}C]	nM	%[^{14}C]	%dose	%[^{14}C]	%dose	%dose
M556/1	35.4	0.3	48.3	0.2	17.3	1.2	44.0	ND [e]	ND	5.7	1.7	1.7
M482/2	40.9	1.1	177	4.7	406	5.0	184	2.5	1.5	4.3	1.3	2.8
M482/1	41.4	2.1	338	16.9	1460	8.7	319	34.2	21.0	38.2	11.3	32.3
M626/1	48.7	0.2	32.2	0.2	17.3	0.2	7.3	ND	ND	0.6	0.2	0.2
M478/1	51.2	0.4	64.4	1.0	86.4	2.4	88.1	1.0	0.6	0.3	0.1	0.7
M468/1+ M380/1 [c, d]	52.1	1.4 [d]	225 [d]	4.5	389	4.0	147	11.5	7.1	7.3 [d]	2.2 [d]	9.3 [d]
M626/2	52.4			0.2	17.3	ND	ND	ND	ND			
M464/2	55.2	1.6	258	3.7	320	3.3	121	3.4	2.0	2.1	0.6	2.6
M464/3	55.8	ND	ND	ND	ND	0.9	33.0	0.7	0.4	ND	ND	0.4
M464/1	56.7	1.4	225	1.2 [d]	104 [d]	2.1 [d]	77.1	11.6	7.1	4.0 [d]	1.2 [d]	8.3 [d]
M626/3	57.0	1.2	193					ND	ND			
Empagliflozin	58.3	88.7	14300	63.6	5500	73.2	2690	31.8	19.5	32.6	9.6	29.1
Total		98.4	15900	96.2	8310	101	3710	96.7	59.3	95.1	28.2	87.5

a % of dosed radioactivity.
b % of sample radioactivity.
c Authentic standard EX 609 for M380/1 was available.
d Metabolites co-eluted and the results are combined.
e Not detected.

Table 26. Empagliflozin Metabolite Profile in Dog, Plasma, Feces and Urine - Female

Oral dose of 5 mg/kg [^{14}C]empagliflozin in female beagle dog												
Compounds	RT	Dog Plasma						Feces + Urine (89.4% of dose) [a]				
		1hr		4hr		8hr		Feces (69.8% dose)		Urine (19.6% dose)		Total
		%[^{14}C] [b]	nM	%[^{14}C]	nM	%[^{14}C]	nM	% [^{14}C]	% dose	%[^{14}C]	% dose	% dose
M556/1	35.4	0.3	46.5	0.8	66.4	0.7	22.5	ND [e]	ND	4.6	0.9	0.9
M482/2	40.9	1.3	202	3.9	324	5.0	161	5.2	3.6	6.4	1.3	4.9
M482/1	41.4	3.0	465	9.1	755	10.2	328	36.5	25.5	34.9	6.8	32.3
M626/1	48.7	0.1	15.5	0.1	8.3	0.1	3.2	ND	ND	0.2	<0.1	<0.1
M478/1	51.2	0.4	62.0	0.6	49.8	1.0	32.2	1.1	0.8	0.5	0.1	0.9
M468/1+ M380/1 [c, d]	52.1	0.8	124	2.7	224	3.2	103	10.2	7.1	5.6	1.1	8.2
M626/2	52.4	0.2	31.0	0.6	49.8	1.4	45.1	ND	ND	0.6	0.1	0.1
M464/2	55.2	0.8	124	3.6	299	2.0	64.4	3.6	2.5	1.6	0.3	2.8
M464/3	55.8	ND	ND	ND	ND	1.4	45.1	2.3	1.6	0.4	0.1	1.7
M464/1	56.7	1.0	155	0.8	66.4	2.1	67.6	15.7	11.0	3.8	0.7	11.7
M626/3	57.0	1.4	217	1.2	99.6	1.5	48.3	ND	ND	0.8	0.2	0.2
Empagliflozin	58.3	86.0	13300	74.2	6160	66.8	2150	23.3	16.3	36.2	7.1	23.4
Total		95.3	14700	97.6	8100	95.4	3070	98.4	68.7	95.6	18.7	87.4

a % of dosed radioactivity.
b % of sample radioactivity.
c Authentic standard EX 609 for M380/1 was available.
d Metabolites co-eluted and the results are combined.
e Not detected.

In the dog, nephritis and nephropathy were identified at high exposure multiples in nonclinical studies ranging from 2 weeks to 12 months, except in the 26-week dog study. Although, the high dose in both the 26 week and 12 month dog studies was 100 mg/kg, the exposure in the 12 month dog study was significantly greater (219-261x MRHD) relative to the 26 week dog study (136-146x MRHD) and this lower exposure in the shorter duration study may explain the lack of the renal findings. Exposure to empagliflozin at 217-289x MRHD in the 13 week dog study also resulted in nephritis and nephropathy.

Nephritis and nephropathy occurred in these dog studies without a defined mechanism and could be mediated by an unstable aldehyde metabolite (described above for the mouse) or other as yet, unidentified reactive intermediates. As oxidative metabolism is a minor pathway for empagliflozin in humans, it is unlikely to produce renal toxicity in humans.

Excretion

Excretion of a single oral dose of [^{14}C]empagliflozin detected as drug-related activity was recovered in predominantly in the feces in the mouse, rat and dog (61 - 82%) followed by the urine (4 – 30%). Total recovery of radioactivity was 95-96%, 72-83% and 87-88% in the mouse, rat and dog, respectively. Empagliflozin was the major component (% of the dose) in the feces for the mouse (64-68%), rat (49-69%) but not the dog (16-20%). In a single dose human study with [^{14}C]empagliflozin at 50 mg, 54% and 41% of the dose was excreted in the urine and feces, respectively. Unchanged empagliflozin also represented 83% and 44% of the fecal and urinary radioactivity.

Further mass balance studies in mice and rats with empagliflozin at the high dose used in the carcinogenicity studies again showed feces as the major route of elimination in both species, followed by urinary excretion (see sponsor's tables below).

Table 27. Excretion of Radioactivity (%) in Male and Female Mice Following Oral Administration of 1000 mg/kg [^{14}C]Empagliflozin

Route of excretion	Time period [h]	male	female	both
urinary	0 - 24	14.0	18.4	16.2
	0 - 96	15.4	19.9	17.7
faecal	0 - 24	73.9	71.4	72.6
	0 - 96	81.7	79.4	80.6
(cage wash)	0 - 96	3.0	3.9	3.5
Total recovery	0 - 96	100.1	103.3	101.7

Table 28. Excretion of Radioactivity (%) in Male and Female Rats Following Oral Administration of 700 mg/kg [^{14}C]Empagliflozin

Route of excretion	Time period [h]	Excretion [% of dose]		
		male	female	male & female mean ± SD
urinary	0 - 48	3.5	7.1	5.3 ± 2.5
	0 - 168	3.5	7.2	5.4 ± 2.5
faecal	0 - 48	78.5	60.6	69.5 ± 17
	0 - 168	79.7	69.4	74.6 ± 14
(cage wash)	0 - 168	0.2	1.0	0.6 ± 0.5
Carcass		<0.04	<0.04	<0.04
Total recovery	0 - 168	83.4	77.6	80.5 ± 14

Empagliflozin Excretion in Rat Milk: Empagliflozin was excreted in the milk of lactating rats at a milk:plasma ratio of 0.635 – 5 (see sponsor's table below). The mean maximal milk:plasma ratio of 5 occurred at 8 hours post-dose, suggesting accumulation of empagliflozin in the milk.

Table 29. Blood: Plasma and Milk: Plasma Concentration Ratios Following 5 mg/kg [^{14}C]-BI 10773 XX in the Female Lactating Rat

Collection Time Point (Hours)	Concentration Ratios: Animal Number 1	2	3	Mean	SD
		Blood:Plasma			
1[a]	0.627	0.645	0.652	0.641	0.013
2[b]	0.659	0.655	0.646	0.654	0.007
4[c]	0.659	0.718	0.667	0.681	0.032
8[d]	0.655	0.651	0.683	0.663	0.017
24[e]	NA	0.639	0.723	0.681	NA
		Milk:Plasma			
1[a]	0.495	0.705	0.701	0.634	0.120
2[b]	2.03	1.32	1.22	1.52	0.44
4[c]	2.39	4.74	2.32	3.15	1.38
8[d]	4.87	1.95	8.20	5.00	3.13
24[e]	0.924	0.699	1.55	1.06	0.44

NA Not applicable.
SD Standard deviation.

5.2 Toxicokinetics

Dedicated repeat dose toxicokinetic (TK) studies were conducted in pregnant rats and rabbits at identical doses used in the definitive embryo-fetal development studies. All studies were fully reviewed and the TKs are summarized here.

Pregnant Rat (GLP study# 07R029, U08-3562-01)
Pregnant dams were treated with empagliflozin by oral gavage from GD 6-17 at 0,100, 300 and 700 mg/kg. Blood was collected at 0 (pre-dose), 1, 2, 4, 8 and 24 hours post-dose at GD 6 and 17. Plasma TK results for empagliflozin are shown in the sponsor's table below.

Table 30. Pregnant Rat TK Summary

Toxicokinetic Parameters of BI 10773 XX after Oral Administration of BI 10773 XX on Drug Days 1 and 12 of a Range-Finding Embryo/Fetal Developmental Toxicity Study in Pregnant Wistar Rats.					
Parameter	Drug Day	BI 10773 XX (mg/kg/day)			
		30	100	300	700
C_{max} (nM)	1	2,860	14,900	24,800	35,300
	12	2,750	17,000	35,000	61,900
AUC_{0-24} (nM•h)	1	14,700	98,000	210,000	275,000
	12	18,800	102,000	228,000	734,000
t_{max} (h)	1	2	2	4	1
	12	1	1	4	1

Pregnant Rabbit (GLP study# 07R031, U08-3555-01)

Pregnant dams were treated with empagliflozin by oral gavage from GD 7-20 at 0,100, 300 and 700 mg/kg. Blood was collected at 0 (pre-dose), 1, 2, 4, 8 and 24 hours post-dose at GD 7 (TK day 1) and 20 (TK day 14). Plasma TK results for empagliflozin are shown in the sponsor's table below.

Table 31. Pregnant Rabbit TK Summary

Toxicokinetic Parameters of BI 10773 XX after Oral Administration of BI 10773 XX on Drug Days 1 and 14 of a Range-Finding Embryo/Fetal Developmental Toxicity Study in Pregnant Rabbits					
TK Parameter	Drug Day	BI 10773 Dose (mg/kg/day)			
		30	100	300	700
C_{max} (nM)	1	10,600	36,400	69,000	68,100
	14	10,000	30,600	77,800	52,800
AUC_{0-24} (nM•h)	1	41,300	188,000	512,000	741,000
	14	37,300	191,000	608,000	659,000
t_{max} (h)	1	1	2	2	2
	14	1	1	2	4

6 General Toxicology

6.1 Single-Dose Toxicity

Single dose toxicity studies were conducted in CD-1 mice and Wistar rats. Empagliflozin was administered by oral gavage (p.o.) or intra-peritoneally (i.p.). A summary of these studies is reported below.

Treatment of mice with empagliflozin intraperitoneally (i.p.) at 2000 mg/kg resulted in mortality for 3 high dose females. As this finding was associated with red fluid-filled intestines in 2/3 females, it likely the mortality of these animals was related to a dosing accident. No histopathology findings were noted in the other 2000 mg/kg female. Clinical signs at 2000 mg/kg included prostration, which is usually considered adverse. The lack of mortality and clinical signs at 300 mg/kg render this dose as the study NOAEL (see table 32 below).

The per os (p.o) single dose study conducted in CD-1 mice and both p.o. and i.p. single dose studies conducted in Wistar rats did not result in adverse findings, and the LD_{50} in these studies is ≥ 2000 mg/kg (see table 32 below). Exposure was not assessed in these mice and rats.

Table 32. Summary of Single Dose Toxicology Studies

SINGLE DOSE TOXICOLOGY STUDIES			
SPECIES/ STUDY	**NOAEL**	**MULTIPLE OF MRHD (25 mg: 4740 nmol.hr/mL)**	**FINDINGS**
Mouse, CD-1 2000 mg/kg, p.o. GLP study # 06R109, U07-3238	2000 mg/kg	n.a.	Loose feces in females at day 2 post-dose. The LD_{50} of empagliflozin is ≥2000 mg/kg. No exposure data.
Mouse, CD-1 300 and 2000 mg/kg, i.p. GLP study # 06R114, U07-3242	300 mg/kg	n.a.	2000 mg/kg: Death of 2/3 females at day 4 post-dose and 1 female at day 5 post-dose. Fluid filled intestines (red) were found in 2/3 females at necropsy. 2000 mg/kg: ↓activity, ↓body tone, abnormal gait, abnormal stance, piloerection, prostration, eyes shut and yellow wet fur (urogenital). No exposure data.
Rat, Wistar 2000 mg/kg, p.o. GLP study # 06R108, U07-3234	2000 mg/kg	n.a.	The LD_{50} of empagliflozin is ≥2000 mg/kg 2000 mg/kg: abnormal gait, yellow stained fur, soft stool/loose stool and poor grooming. No exposure data.
Rat, Wistar 300 and 2000 mg/kg, i.p. GLP study # 06R1115, U07-3241	2000 mg/kg	n.a.	The LD_{50} of empagliflozin is ≥2000 mg/kg 300 mg/kg: abnormal gait, abnormal stance, soft stool/loose stool and ↓activity. 2000 mg/kg: piloerection, soft stool/loose stool and poor grooming. No exposure data.

n.a. – not applicable

6.2 Repeat-Dose Toxicity

General toxicity was assessed in Wistar (Han) rats and Beagle dogs in GLP studies up to 6 months and 12 months in duration, respectively. Exposure to empagliflozin ranged from 2x to 78x the MRHD in the rat and 13x to 289x the MRHD in the dog, respectively.

In addition, a 3 month toxicity study was conducted in CD-1 mice from 27x to 98x MRHD. All studies were reviewed and are summarized below.

Mice: A 13-Week Oral Gavage Toxicity and Toxicokinetic Study in the CD-1 Mouse with BI 10773 XX
0, 500, 750 and 1000 mg/kg
Exposure margins:
M: 27x, 47x and 62x MRHD
F: 77x, 68x and 99x MRHD

Key Findings

- Mortality occurred in two males at 1000mg/kg/day, but was not related to empagliflozin treatment.
- Abdominal (males and females) and dermal swelling (males only) occurred in all treated animals. Hair loss was noted in the HD males and all treated females.
- Food consumption was increased at all doses.
- Liver weight was slightly increased at all doses in males and females (~24%); kidney weight was increased in females (~23%).
- Target organs of toxicity were the kidney (single cell necrosis, tubular karyomegaly, cystic tubular hyperplasia, interstitial nephritis and chronic nephropathy), liver (mid-zonal hydropic change) and testis (tubular degeneration, unilateral). However, the toxicity in the liver and kidney did not correlate with changes in liver function tests (LFTs) or kidney biomarkers (creatinine and BUN).
- Immunohistochemical staining of the kidney with the proliferation marker Ki-67 showed exacerbation of staining and proliferation in the renal tubular epithelial cells (proximal convoluted tubules) in the superficial cortex of the 750 and 1000 mg/kg males. This was accompanied by increased mitoses in the same area.
- Due to a lack of frank toxicity, the NOAEL is 1000 mg/kg which is 62x and 98x MRHD in males and females, respectively.

Rat: 2 Week Oral (Gastric Intubation) Range-Finding Toxicity Study in the Rat with BI 10773 (BIPI Study No. 05R214)
0, 30, 100, 300 and 500 mg/kg

Key Findings
- Loose stool and diarrhea at ≥ 300 mg/kg/day.
- Significant decrease in BW gain at 300 (-16% M) and 500 mg/kg/day (-37% M, -13% F).
- ↓ Serum glucose in male at ≥ 30 mg/kg/day and females ≥100 mg/kg/day, respectively.
- ↑ Serum BUN in males at ≥ 30 mg/kg/day and females ≥ 100 mg/kg/day (dehydration and protein break down was suspected).
- Dose-dependent ↑ in triglycerides in male and females.
- ↑ Urine volume in females at ≥ 30 mg/kg/day.
- ↓ Urine pH and ↑ in ketone bodies in males ≥ 100 mg/kg/day
- ↑ Urinary glucose at all doses (≥ 1000 mg/dl)
- Subacute pyelonephritis and cystitis in control females and MD and HD females.
- The NOAEL is 100 mg/kg which is approximately 2x MRHD in males and females, respectively.

Rat: A 4-Week Subchronic Toxicity Study of BI 10773 XX Administered by the Oral (Gavage) Route to Wistar Rats with a 4-Week Recovery Period
0, 30, 100 and 1000 mg/kg
Exposure margins:
M: 1x, 3x and 45x MRHD
F: 1x, 4x and 69x MRHD

Key Findings
- There were 2 deaths (1 control male and 1 HD female).
- The death of HD female (69x MRHD) was associated with bilateral pyelonephritis and marked chronic active transmural inflammation in the urinary bladder (likely drug-related).
- ↑ Food intake at both the MD (12-15%) and HD (35%).
- Dose-dependent ↓ serum glucose in all doses.
- Slight ↑ in AST, ALT and ALP at ≥ 100 mg/kg/day without pathology correlates.
- ↑ in serum globulin, triglycerides and BUN at ≥ 100 mg/kg/day
- ↓ serum chloride and bilirubin
- ↑ urine glucose and polyuria at ≥ 100 mg/kg/day
- ↑ urinary ketones at 100 mg/kg/day likely due to break down of fat.
- ↑ kidney weight in MD (8.9%) and HD (25%) females.
- ↓ salivary gland weight in MD females (10%) and HD (12%) male and females.
- Due to the uncertain nature of the female HD death, the NOAEL is 100 mg/kg which is 3x and 4x MRHD in males and females, respectively.

Rat: Thirteen-Week Oral (Gastric Intubation) Toxicity Study in the Rat with BI 10773 XX Followed by a Four-Week Recovery Period
0, 30, 100 and 700 mg/kg
Exposure margins:
M: 3x, 11x and 44x MRHD
F: 6x, 11x and 77x MRHD

Key Findings
- One HD female rat was found dead on week 11. The cause of death was not determined.
- Clinical signs noted only in the HD were soft stool and diarrhea.
- No notable change in body weight at any dose level.
- Increased food intake was noted at all doses in males and also the MD and HD females.
- Slight hematology changes in HD rats (↓ WBC, lympocytes in males, and RBC, Hct and Hgb in HD females).
- ↓Serum glucose at ≥ 100 mg/kg/day
- ↑BUN at ≥ 100 mg/kg/day
- ↑AST and ↑ALT at all doses (< 2 fold & not dose-related).
- ↑Triglycerides at 700 mg/kg/day
- ↓Serum sodium at ≥ 30 mg/kg/day), ↓Chloride at ≥ 100 mg/kg/day.
- ↑ Serum phosphate (111 to 122% of control) at 700 mg/kg/day. Hyper-phosphatemia has been known to cause chronic renal disease
- ↑ Urine Glucose and polyuria at all doses and ketone bodies at ≥ 100 mg/kg/day
- Dose-relate increase in renal tubular, papillary and pelvic mineralization (≥ 100 mkd).
- Renal mineralization also found in recovery animals.
- The NOAEL is 100 mg/kg/day which is 11x MRHD in males and females, respectively.

Rat: A 6-Month Oral (Gavage) Toxicity Study in Rats With a 3-Month Recovery Period
0, 30, 100 and 700 mg/kg
Exposure margins:
M: 2x, 10x and 35x MRHD
F: 5x, 19x and 78x MRHD

Key Findings
- ↑ Urine Glucose at all doses and polyuria at ≥100 mg/kg.
- One 700 mg/kg/day male and female was found dead at study day 38 and 8, respectively. No microscopic cause of death was identified.
- At the end of treatment the mean body weight was decreased 21% in the 700 mg/kg/day males.
- Mild lymphopenia was observed in 100 and 700 mg/kg/day males. In the thyroid gland, minimal follicular cell hypertrophy was noted in all treated males.

- A low incidence (3/12) of hepatocellular necrosis was observed in 700 mg/kg/day males that correlated microscopically with lipid containing vacuoles. Hepatocellular vacuolation occurred at all doses.
- A dose dependent increase and exacerbation of kidney tubular and cortical mineralization was observed at all doses of drug. Corticomedullary tubular dilation and vacuolation was noted in the 700 mg/kg animals and the 100 mg/kg females. The vacuoles were found to contain lipid. Renal mineralization was not reversed in 700 mg/kg/day animals.
- Vacuolation of the adrenal zona glomerulosa and hypertrophy of the zona fasiculata was observed in the all empagliflozin-treated males and females with increasing incidence and severity in the high dose animals.
- Key PD markers urinary calcium and phosphate were not measured.
- The NOAEL was not established due to vacuolation in all treatment groups for the adrenal (minimal to moderate), liver (minimal to marked) and kidney (≥100 mg/kg) (minimal to mild) with no vacuolation findings in the control animals. Empagliflozin at 700 mg/kg is 35x and 78x MRHD in males and females, respectively.

Table 33. 6 Month Rat Target Organ Histology

Daily Dose (mg/kg/day)	0 (Control)		30		100		700	
Drug Phase								
Number of Animals	M: 12	F: 12	M: 12	F: 12	M: 12	F: 12	M: 12	F: 2
Kidneys Cortical Tubular Vacuolation								
Minimal	-	-	-	-	-	3	-	9
Mild	-	-	-	-	-	-	1	1
Adrenals Vacuolation:Zona Glomerulosa								
Minimal	-	-	10	12	9	10	5	7
Mild	-	-	1	-	3	-	7	3
Moderate	-	-	-	-	-	-	1	-
Hypertrophy: Zona Fasciculata								
Minimal	-	-	5	6	8	12	4	6
Mild	-	-	2	1	-	-	2	3
Moderate	-	-	-	-	-	-	-	2
Liver Microvesicular Hepatocellular Vacuolation								
Minimal	-	-	2	4	2	4	-	3
Mild	-	-	-	6	1	2	3	2
Moderate	-	-	-	1	-	3	1	6
Marked	-	-	-	-	-	-	-	1
Pancreas Vacuolation: Acinar Epithelial Cells								
Minimal	-	-	-	-	1	-	6	1
Mild	-	-	-	-	1	-	4	2
Thyroid Follicular Cell Hypertrophy								
Minimal	-	-	2	-	2	-	3	-

Table 34. 6 Month Rat: Incidence and Severity of Renal Mineralization

Daily Dose (mg/kg/day)	0 (Control)		30		100		700	
Drug Phase								
Number of Animals	M: 12	F: 12	M: 12	F: 12	M: 12	F: 12	M: 12	F: 2
Kidneys								
Cortical								
Tubular								
Mineralization	5	6	1	4	2	2	1	3
Minimal	-	3	8	1	7	7	10	9
Mild								
Papillary								
	2	3	7	3	7	4	9	8
Mineralization	-	1	-	1	-	-	-	2
Minimal								
Mild								
Recovery Phase								
Number of Animals	M: 5	F: 5	N/A	N/A	N/A	N/A	M: 5	F: 5
Kidneys								
Cortical								
Tubular								
Mineralization	1	-					-	2
Minimal	-	1					3	3
Mild	-	-					1	-
Moderate								
Papillary								
	1	-					2	2
Mineralization								
Minimal								

Table 35. 6 Month Rat TK Data

Toxicokinetic Parameters of BI 10773 after Oral Administration of BI 10773 XX to Rats					
TK Parameter	Gender	Drug Day	BI 10773 XX (mg/kg/day)		
			30	100	700
C_{max} (nM)	Male	Day 0	1,070	12,400	12,400
		Day 32	1,420	7,490	31,700
		Day 95	1,220	7,400	14,800
		Day 178	1,300	9,200	14,000
	Female	Day 0	2,170	12,700	35,500
		Day 32	1,550	12,000	45,600
		Day 95	2,170	10,400	29,500
		Day 178	5,050	20,200	40,400
AUC_{0-24} (nM•h)	Male	Day 0	7,430	41,500	96,300
		Day 32	8,830	44,900	217,000
		Day 95	8,280	56,400	126,000
		Day 178	11,200	47,400	166,000
	Female	Day 0	8,550	48,100	216,000
		Day 32	10,200	77,600	422,000
		Day 95	7,650	60,300	336,000
		Day 178	26,000	87,800	372,000
t_{max} (h)	Male	Day 0	2	2	4
		Day 32	2	2	4
		Day 95	2	4	2
		Day 178	4	2	2
	Female	Day 0	2	2	4
		Day 32	2	2	4
		Day 95	2	4	4
		Day 178	4	2	2

Dog: 2-Week Oral (Gastric Intubation) Range-Finding Toxicity Study in the Beagle Dog with BI 10773 (BIPI Study Number 05R215)
0, 30, 100, 300 and 500 (200) mg/kg
Exposure margins:
M: 35x, 128x, 314x and 157x MRHD
F: 38x, 161x, 231x and 292x MRHD

Key Findings
- Substantial BW loss at 500 mg/kg/day (8-14%) resulted in HD dose reduction to 200 mg/kg/day on Day 8 (dogs appeared emaciated).
- Diarrhea and soft stool was noted at ≥ 100mg/kg/day.
- Decreased in food intake in females at ≥ 100 mg/kg/day
- BW was reduced in all animals at ≥ 100 mg/kg/day.
- Dose dependent increase in Hct at ≥ 30 mg/kg/day.
- ↓ Serum glucose ≥ 100 mg/kg/day
- ↑ Urinary glucose at all doses.
- ↑ BUN in females ≥ 100 mg/kg/day and males at 500 mg/kg/day
- ↑ Serum creatinine and Ca at ≥ 500 (200) mg/kg/day.
- Subacute interstitial nephritis and tubular nephropathy at ≥ 100 mg/kg/day.
- Glandular atrophy of prostate at 500 mg/kg/day in dogs. This was likely due to BW loss.
- Calcification present in renal papillae in female at 300 mg/kg/day
- Calcification present in aorta and heart chordae tendineae in females at 500 (200) mg/kg/day. These findings were no seen in the 4 and 13 week dog studies (lower doses)
- The NOAEL is 30 mg/kg which is 35x and 38x MRHD in the male and female, respectively.

Dog: A 28-Day Toxicity Study of BI 10773 Administered by the Oral (Gavage) Route to Dogs with an 8-Week Recovery Period
0, 10, 30, 100 mg/kg
Exposure margins:
M: 7x, 13x and 73x MRHD
F: 6x, 13x and 69x MRHD

Key Findings
- Soft stool and diarrhea was noted at ≥ 30 mg/kg/day.
- Dose-dependent decrease in BW at the end of the study that was significant in HD males (dogs appeared emaciated).
- ↑ Urinary glucose (moderate to high) at all empagliflozin doses.
- ↑ Urine volume at all doses on Day 11.
- Subacute interstitial nephritis and tubular nephropathy in 1 HD male and female. Minimal chronic interstitial nephritis in 1 recovery HD male.
- Glandular atrophy of prostate in 1 HD (moderate) and 2 MD (slight) may have been due to weight loss/emaciation

- The NOAEL is 10 mg/kg/day which is 7x and 6x MRHD in males and females, respectively.

Dog: Thirteen-Week Oral (Gastric Intubation) Toxicity Study in the Beagle Dog with BI 10773 XX Followed by a Thirteen Week Recovery Period

0, 10, 30, 100 mg/kg
Exposure margins:
M: 15x, 60x and 217x MRHD
F: 24x, 74x and 289x MRHD

Key Findings

- Dose-dependent increase in soft stool and diarrhea at ≥ 30 mg/k/day. MD and HD dogs appeared thin.
- MD and HD dogs appeared dehydrated starting at Wk 4 and early in the recovery (Wk 2)
- There were no significant differences in BW at the end of the study, however, BW was reduced at the beginning of the treatment (↓ BW at 30 mg/kg/day in females and both sexes at 100 mg/kg/day up to Wk 9). The HD dose group was supplemented with high calorie canned food (beginning Wk 6).
- Several hematology parameters were slightly altered (↑ platelets by 2 fold at ≥ 30 mg/kg/day, ↑ Hgb in HD males, ↓ Lymphocytes in HD females)
- ↓ serum chloride at 100 mg/kg/day
- ↓ serum Glucose in both sexes at all doses
- ↑ Urinary Glucose at Wk 4 and 13
- ↑ Urine volume at all doses at Wk 4 and 13
- ↑ incidence of RBC in urine at ≥ 30 mg/kg/day suggesting excess stress on kidneys and suggesting renal injury
- ↓ urinary pH at 100 mg/kg/day at Wk 4 and 13
- Recovery hematology and clinical chemistry parameters were unremarkable.
- Chronic interstitial nephritis and cortical tubular nephropathy in 1 MD female only. It is not clear if supplementing HD dogs with high calorie canned food had any influence on renal findings in HD dogs.
- Increase in panlobular hepatocellular vacuolation marked by Oil Red O lipid droplets in 1 MD male and 3 males and 2 female in the HD dogs
- Dose-dependent decrease in hepatocellular cytoplasmic glycogen at all doses in both sexes
- Hypertrophy of Kupffer's cells in the liver of 1 HD female
- Atrophy of glandular prostate in HD males.
- Multifocal chronic tubulo-interstitial nephritis was noted in 1 HD recovery male. Since similar findings were seen in the 4-wk dog study, it was considered drug-related and not reversible.
- Due to no frank toxicity the NOAEL is 100 mg/kg/day which is 217x and 289x MRHD in males and females, respectively.

Dog: A 26-Week Toxicity Study of BI 10773 XX Administered by Oral Gavage to Dogs with a 13-Week Recovery Period
0, 10, 30 and 100 mg/kg
Exposure margins:
M: 15x, 54x and 136x MRHD
F: 12x, 48x and 146x MRHD

Key Findings
- Dose-dependent increase in diarrhea with high incidence in 100 mg/kg animals that resulted in reduced BW and BW gain. Also, reduced BW gain for the mid and high dose animals. High dose animals required supplementation with high calorie canned food.
- ↑ Urinary Glucose at all doses and polyuria at 100 mg/kg.
- The liver to body weight ratio were increased in the high dose males (↑30%) and the mid (↑14%) and high dose (↑21%) females, respectively. Microscopically minimal to mild hepatic vacuolation, hepatocellular degeneration and Kupffer cell hypertrophy was noted in the 100 mg/kg/day-treated animals.
- Hypocellularity of the bone marrow was observed in mid and high dose animals. Hematology was not adversely affected.
- Creatinine was statistically significantly decreased in the high dose males (↓20%) and females (↓25%).
- Key PD markers urinary calcium and phosphate were not measured.
- The mean serum glucose was statistically significantly decreased in the mid (↓21%) and high (↓24%) dose males and all treated females (↓20-28%). Urinary glucose was increased in the empagliflozin-treated males and females.
- The NOAEL is 10 mg/kg which is 10 mg/kg which is 15x and 12x MRHD in males and females, respectively

Dog: A 52-Week Toxicity Study of BI 10773 XX Administered by Oral Gavage to Dogs with a 13-Week Recovery Period
0, 10, 30 and 100 mg/kg
Exposure margins:
M: 19x, 55x and 219x MRHD
F: 17x, 50x and 261x MRHD

Key Findings
- Creatinine was statistically significantly decreased in the high dose males (↓20%) and females (↓25%).
- Mortality observed in one the 30 mg/kg/day males.
- ↑ Urinary Glucose at all doses.
- There was a dose-dependent increase in diarrhea and soft stools at all doses with a markedly increased incidence at the mid and high dose animals.
- Final body weight was reduced at all doses with a dose-dependence (10% to 31% in males, and 15% to 27% in females).

- The absolute heart weights and the heart to brain weight ratios were statistically significantly (SS) reduced in the 30 and 100 mg/kg/day males and also the 10 and 100 mg/kg/day females, respectively; but were without pathology correlates.
- A dose related increase in the severity of vacuolation of the adrenal zona glomerulosa was observed at the mid and high dose groups (from minimal to mild/moderate). Nearly all animals in all dose groups had baseline minimal vacuolation.
- Nephritis and cortical tubular degeneration with fibrosis was noted in high dose animals.
- Bone biomarkers osteocalcin and bALP were increased in the mid and high dose animals without bone histopathology.
- Key PD markers urinary calcium and phosphate were not measured.
- The NOAEL is 30 mg/kg which is 55x and 50x MRHD in males and females, respectively.

Table 36. 52 Week Dog Target Organ Histology - Males

MALE HISTOPATHOLOGY, 12 MONTHS WITH 13-WEEK RECOVERY						
Tissue	**Finding**	**Severity**	**0**	**10**	**30**	**100**
			n=6	**n=6**	**n=6**	**n=6**
Liver	Vacuolation, panlobular	Moderate				1
Kidney	Nephritis, interstitial	Minimal				2
		Mild				3
Kidney	Atrophy, cortical tubular	Minimal				3
		Mild				
		Moderate				
Testis	Atrophy	Moderate				1
Adrenal	Vacuolation, zona glomerulosa	Minimal	3	5	3	3
		Mild				
		Moderate			1	1
Tissue	**Finding**	**Severity**	**0**	**10**	**30**	**100**

Table 37. 52 Week Dog Target Organ Histology - Females

FEMALE HISTOPATHOLOGY, 12 MONTHS WITH 13-WEEK RECOVERY						
Tissue	**Finding**	**Severity**	**0**	**10**	**30**	**100**
			n=6	**n=6**	**n=6**	**n=6**
Kidney	Nephritis, interstitial	Minimal				1
		Mild				1
Kidney	Nephropathy, cortical tubule	Minimal				1
		Mild				
		Moderate				1
Adrenal	Vacuolation, zona glomerulosa	Minimal	4	3	4	4
		Mild			1	1
		Moderate				
Tissue	**Finding**	**Severity**	**0**	**10**	**30**	**100**

Table 38. Dog 52 Week Toxicokinetics Data

Dose Level (mg/kg/day)	Gender	C_{max} (nM)	$AUC_{0\text{-}24}$ (nM·h)	t_{max} (h)[a]
Day 1				
10[b]	Male	7640	65,500	2 (1-4)
	Female	8370	71,100	2 (1-2)
30[b]	Male	23,400	225,000	2 (1-2)
	Female	24,100	231,000	2 (1-2)
100[c]	Male	74,200	829,000	2 (2-8)
	Female	86,200	1,030,000	2 (2-4)
Day 364				
10[b]	Male	11,000	88,900	2 (1-2)
	Female	9490	82,800	2 (2-2)
30[b]	Male	33,000[d]	262,000[d]	1 (1-2)
	Female	26,600	238,000	2 (1-2)
100[c]	Male	97,800	1,040,000	3 (2-4)
	Female	113,000	1,240,000	2 (1-8)

[a] t_{max} reported as median (range).
[b] N = 6 per sex.
[c] N = 8 per sex.
[d] N = 5 due to the death of dog #D2411.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

BI 10773 XX: Mutagenicity Testing with Salmonella Typhimurium TA1535, TA1537, TA98 and T100 and Escherichia coli WP2 uvrA (pKM 101). Plate Incorporation Reverse Mutation Assay with and without Metabolic Activation

Study no.:	06R089, U07-3186
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharmaceuticals Inc. 900 Ridgebury Road Ridgefield, CT 06877, USA
Date of study initiation:	June 7th 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 10773 XX, BI10773XXA06 and 98.8%

Key Study Findings

Empagliflozin was tested in an Ames assay using S. Typhimurium and E. Coli strains with and without S9, up to a concentration of 5000 μg/plate. Empagliflozin did not induce a dose-dependent increase in histidine and tryptophan revertants and therefore shows no evidence of mutagenic potential.

Methods

Strains:	S. Typhimurium: TA1535, TA1537, TA98 and TA100 E.Coli: WP2 uvrA (pKM101)
Concentrations in definitive study:	156, 313, 625, 1250, 2500 and 5000 μg/plate.
Basis of concentration selection:	Tested at up to the limit of solubility (precitpitate that prevented plate reading) or excessive toxicity on reduced background lawn.
Negative control:	DMSO
Positive control:	-S9: Sodium Azide, 2-NF, 9-AA. +S(9): 2-AA
Formulation/Vehicle:	DMSO
Incubation & sampling time:	48 hr

Study Validity

Study was valid. Dose selection for the plate incorporation assay was adequate based on the limit dose of 5000 μg/plate. Empagliflozin was tested in triplicate cultures and the positive controls gave the expected results.

Results

When tested to a maximum concentration of up to 5000 μg/plate, empagliflozin was not mutagenic in the presence or absence of S9 (sponsor's tables below).

Table 39. Mean Revertant Counts

Genotoxicity: **In Vitro** **Report Title:** BI 10733 XX: Mutagenicity testing with *Salmonella typhimurium* TA1535, TA1537, TA98, TA100 and *Escherichia coli* WP2 uvrA (pKM101). Plate incorporation reverse mutation assay with and without metabolic activation (Study No. 06R089) **Test Article:** BI 10773 XX

Test for Induction of: Reverse mutation in bacterial cells
Strains: S. typhimurium and E. coli
Metabolizing System: Aroclor 1254 induced rat liver S9, 50 μl/plate
Vehicles: For Test Article: DMSO
Treatment: Plate incorporation method + 48 hr on plates.
Cytotoxic Effects: Reduced number of TA100 colonies observed with 5000 μg/plate BI 10733 XX with and without S-9
Genotoxic Effects: None
No. of Independent Assays: 2
No. of Replicate Cultures: 2
No. of Cells Analyzed/Culture: N/A
For Positive Controls: DMSO/MilliQ water
Report No.: U07-3186
Location in Module 4: 4.2.3.3.1
GLP Compliance: Yes
Date of Treatment: June 2006

First replicate, June 9, 2006

Metabolic Activation	Test Article	Dose (ug/plate)	Revertant Colony Counts (mean ± SD)				
			TA98	TA100	TA1535	TA1537	E. Coli
Without activation	DMSO	50 μl/plate	11±3	145±21	6±2	4±2	173±6
	BI 10733 XX	156	12±3	152±7	5±2	4±1	178±8
		313	11±2	146±4	6±2	5±1	175±23
		625	10±1	142±13	5±3	6±1	179±16
		1250	7±1	148±11	4±3	5±1	163±25
		2500	13±1	124±11	6±3	3±1	177±19
		5000	12±4	51±13	3±1	6±3	177±17
	Sodium Azide	1					
	9-Aminoacridine	50				388±17	
	2-Nitrofluorine	1	173±15				
	Sodium Azide	10		806±75	819±6		
	MMS	1.25					1368±52
With activation	DMSO	50 μl/plate	11±4	175±14	10±4	2±1	173±10
	BI 10733 XX	156	10±2	183±4	8±2	5±2	201±9
		313	11±1	151±14	9±3	4±1	207±17
		625	10±5	152±3	9±3	4±2	221±10
		1250	16±8	169±11	6±2	5±3	234±9
		2500	12±2	147±14	8±2	4±3	175±14
		5000	16±2	103±12	6±2	7±2	205±16
	2-Amino-anthracene	10					
	2-Amino-anthracene	2				88±17	648±13
	2-Amino-anthracene	1	451±13	935±44	115±7		

Second replicate June 16, 2006

Metabolic Activation	Test Article	Dose (ug/plate)	Repentant Colony Counts (mean ± SD)				
			TA98	TA100	TA1535	TA1537[a]	E. Coli
Without activation	DMSO	50 µl/plate	11±3	141±18	6±1	5±1	165±8
	BI 10733 XX	156	10±3	137±20	7±4	6±0	175±3
		313	10±5	146±45	5±1	5±0	152±8
		625	7±2	147±19	4±2	6±5	175±22
		1250	16±2	129±3	4±3	8±1	172±16
		2500	13±3	147±12	4±1	5±1	160±7
		5000	9±2	60±3	4±2	6±3	168±6
	Sodium Azide	1					
	9-Amino acridine	50				877±397	
	2-Nitro-fluorine	1	201±18				
	Sodium Azide	10		753±37	919±121		
	MMS	1.25					1527±77
With activation	DMSO	50 µl/plate	11±1	157±7	8±1	8±5	174±6
	BI 10733 XX	156	13±1	167±6	10±4	12±4	196±4
		313	17±2	149±12	8±1	10±3	204±15
		625	11±6	178±13	9±1	7±2	212±9
		1250	13±3	172±2	9±2	9±2	204±21
		2500	14±3	149±15	5±1	9±1	192±24
		5000	12±7	129±7	4±2	12±3	209±12
	2-Amino-anthracene	10					
	2-Amino-anthracene	2				122±22	740±31
	2-Nitro-fluorine	1					
	2-Amino-anthracene	1	460±36	907±45	140±126		

a Data for the TA1537 test without activation is from a third replicate necessitated because of an aberrant control response during the second replicate. Data for the TA1537 test with activation is from the second replicate.

7.2 *In Vitro* Assays in Mammalian Cells

BI 10773 XX: Mutagenicity Testing with L5178Y tk+/- Mouse Lymphoma Cells. Forward Mutation Assay

Study no.: 06R088, U07-3245
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 18th July 2006
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BI 10773 XX, BI10773XXA06 and 98.8%

Key Study Findings

Empagliflozin was not mutagenic in a 4hr assay with and without metabolic activation (+/-S9) or in a 24 hr assay without metabolic activation (-S9).

Methods

Cell line: L5178Y tk +/-
Concentrations in definitive study: 4 hr: 200-400 µg/mL, 24 hr: 100-300 µg/mL
Basis of concentration selection: Dose range finding study
Negative control: DMSO

Positive control: +S9: 3-MC (MCA) at 1 μg/mL, -S9: MMS at 7.5 μg/mL
Formulation/Vehicle: DMSO
Incubation & sampling time: 4 hr +/- S9 and 24 hr –S9

Study Validity

Vehicle control cultures were acceptable. The positive controls yielded the expected results. The dose range was acceptable and the limit on empagliflozin concentration was based on cytotoxicity in the dose range finding study and solubility.

Results

In the initial 4 hr treatment with and without S9, there was no significant increase in mutant frequency (see sponsor's tables below).

Table 40. Mutation Assay for 4 hr -S9

Dose Level	Daily Counts ($x10^5$ cells/ml) 1	2	Relative Suspension Growth (% Control)	Average Mutant Clones	Average Viable Clones	Relative Cloning Eff. (% Control)*	% Total Relative Growth**	Mutant Freq. $x10^{-6}$***
Vehicle Control	6.9	11.3	100	14	176	88	100	15.9
	6.6	15.1	100	9	102	51	100	18.4
	7.4	14.5	100	8	120	60	100	12.7
	6.9	15.3	100	10	79	40	100	25.3
200 μg/ml	5.1	13.1	68	11	88	74	50	24.2
	5.3	13.9	76	9	91	76	58	19.8
250 μg/ml	5.2	14.6	78	9	91	76	59	19.8
	4.7	12.5	60	13	94	79	47	27.0
300 μg/ml	4.7	14.3	69	11	102	86	59	20.9
	4.9	12.3	62	11	90	75	47	23.7
350 μg/ml	4.0	12.9	53	12	107	90	48	23.1
	3.9	12.9	40	7	74	62	25	19.9
400 μg/ml	2.8	12.4	38	18	162	136	52	22.7
	3.0	11.6	36	17	126	106	38	26.4
MMS 7.5 μg/ml	6.2	11.8	75	61	64	54	40	189.6
	6.6	10.7	72	63	85	71	52	147.5

* Relative Cloning Efficiency:
Solvent Controls relative to 200 (# cells plated)
Dose Levels: relative to Average Solvent Control Viable Count (119)
** %Total Relative Growth: (Relative Suspension Growth x Relative Cloning Eff.)/100
*** Mutant Frequency: [(Total Mutant Clones)/(Total Viable Clones)x($2x10^{-4}$)]

Mean Vehicle Mutant Frequency = 18.1×10^{-6}

Vehicle Control = DMSO 0.01 ml/ml

Table 41. Mutation Assay Results 4 hr +S9

Dose Level	Daily Counts ($x10^5$ cells/ml) 1	2	Relative Suspension Growth (% Control)	Average Mutant Clones	Average Viable Clones	Relative Cloning Eff. (% Control)*	% Total Relative Growth**	Mutant Freq. $x10^{-6}$***
Vehicle Control	6.5	13.0	100	24	124	62	100	38.6
	7.0	11.4	100	24	167	83	100	28.4
	6.5	12.1	100	25	129	64	100	38.9
	4.2	11.1	100	37	131	66	100	56.3
100 µg/ml	6.5	11.6	105	23	147	107	112	31.3
	6.9	11.8	113	20	156	113	128	25.2
150 µg/ml	7.1	12.1	119	18	108	78	93	33.3
	7.4	12.2	125	22	168	122	153	26.6
200 µg/ml	7.6	12.2	128	21	144	105	134	29.6
	7.6	12.1	126	26	200	145	183	25.7
250 µg/ml	6.5	11.7	105	21	145	105	110	29.0
	5.3	13.1	96	15	149	108	104	20.1
300 µg/ml	2.4	8.6	35	15	161	117	41	18.3
	2.0	9.0	37	23	143	104	39	32.6
MCA 1.0 µg/ml	5.8	12.6	101	84	119	86	88	140.6
	6.4	11.7	103	96	153	111	115	125.2

* Relative Cloning Efficiency:
Solvent Controls relative to 200 (# cells plated)
Dose Levels: relative to Average Solvent Control Viable Count (138)

** %Total Relative Growth: (Relative Suspension Growth x Relative Cloning Eff.)/100

*** Mutant Frequency: [(Total Mutant Clones)/(Total Viable Clones)x($2x10^{-4}$)]

Mean Vehicle Mutant Frequency = 40.6×10^{-6}

Vehicle Control = DMSO 0.01 ml/mL

To confirm the negative results, the study was repeated for 24 hr in the absence of S9 and again there was no significant increase in mutant frequency (see sponsor's table below).

Table 42. Mutation Assay Results 24 hr -S9

Dose Level	Daily Counts ($x10^5$ cells/ml) 1	2	Relative Suspension Growth (% Control)	Average Mutant Clones	Average Viable Clones	Relative Cloning Eff. (% Control)*	% Total Relative Growth**	Mutant Freq. $x10^{-6}$***
Vehicle Control	11.8	8.4	100	17	164	82	100	20.8
	12.5	9.1	100	18	185	93	100	19.5
	11.9	9.7	100	13	178	89	100	15.0
	12.6	7.8	100	21	199	99	100	20.8
100 µg/ml	6.4	7.9	48	10	179	99	47	11.5
	5.8	7.6	41	17	190	105	43	17.9
150 µg/ml	4.5	5.4	23	12	150	82	19	16.0
	4.3	6.5	26	12	137	75	20	17.6
200 µg/ml	3.4	7.1	20	12	115	63	13	20.3
	3.5	6.3	18	7	137	75	13	10.2
250 µg/ml	2.8	6.0	17	8	128	71	12	13.0
	2.4	4.0	11	10	109	60	7	18.9
300 µg/ml	2.0	2.7	8	16	116	64	5	28.2
	2.1	2.6	7	10	124	69	5	15.5
MMS 7.5 µg/ml	8.1	10.9	82	88	30	17	14	584.4
	8.1	11.2	85	94	27	15	12	702.5

* Relative Cloning Efficiency:
Solvent Controls relative to 200 (# cells plated)
Dose Levels: relative to Average Solvent Control Viable Count (181)

** %Total Relative Growth: (Relative Suspension Growth x Relative Cloning Eff.)/100

*** Mutant Frequency: [(Total Mutant Clones)/(Total Viable Clones)x($2x10^{-4}$)]

Mean Vehicle Mutant Frequency = 19.0×10^{-6}

Vehicle Control = DMSO 0.01 ml/ml

L5178Y TK$^{+/-}$ Mouse Lymphoma Forward Mutation Assay with Three Different Conditions

Study no.:	06R223, U08-3198-01
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	25 September 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 10773 XX, BI10773XXA06 and 98.8%

Key Study Findings

Empagliflozin was not mutagenic in a 4hr assay with and without metabolic activation (+/-S9) or in a 24 hr assay without metabolic activation (-S9).

Methods

Cell line:	L5178Y tk +/-
Concentrations in definitive study:	4 hr: 50-400 μg/mL, 24 hr: 12.5-175 μg/mL
Basis of concentration selection:	Dose range finding study
Negative control:	DMSO
Positive control:	+S9: 3-MC at 5 - 10 μg/mL, -S9: MMS at 6.5 - 18 μg/mL
Formulation/Vehicle:	DMSO
Incubation & sampling time:	4 hr +/- S9 and 24 hr –S9

Study Validity

Vehicle control cultures were acceptable. The positive controls yielded the expected results. The dose range was acceptable and the limit on empagliflozin concentration was based on cytotoxicity in the dose range finding study and solubility.

Results

In the initial 4 hr treatment with and without S9, there was no significant increase in mutant frequency (see sponsor's tables below).

Table 43. Mutation Assay for 4 hr -S9

Test Condition	Daily Cell Density/mL (x 10^5)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (x 10^{-6})[d]
	Day 1	Day 2								
Nonactivation Controls[e]				AVG VC				AVG VC		
Vehicle Control	11.9	11.7	15.5		108	527	87.8		106.8	41.0
Vehicle Control	10.6	10.6	12.5		107	517	86.2		84.6	41.4
Vehicle Control	11.2	10.5	13.1	13.7	171	631	105.1	93.0	107.9	54.3
MMS 13 µg/mL	7.6	9.0	7.6		403	239	39.8		23.8	337.0[f]
MMS 18 µg/mL	8.7	7.1	6.9		324	195	32.5		17.6	331.8[f]
Test Article (µg/mL)			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
200	10.8	10.0	87.8		115	398	71.3		62.6	57.5
250	10.9	9.8	86.8		93	517	92.6		80.4	35.9
275	9.3	11.1	83.9		81	459	82.3		69.0	35.2
300	9.4	7.2	55.0		137	600	107.5		59.1	45.8
350	5.7	7.7	35.7		107	384	68.8		24.5	55.7
375	4.8	7.4	28.9		137	404	72.3		20.9	68.1
400	2.7[g]	6.2	15.1		128	400	71.7		10.8	63.8
425	0.4[g]	3.9	9.5		--[h]	243	43.6		4.1	--

[a]RSG = (Day 1 Count/3) x (Day 2 Count)/3 (or Day 1 Count if not subcultured)

[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100

[c]Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100

[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}

[e]Vehicle Control = 1% DMSO
Positive Control: MMS = Methyl methanesulfonate

[f]Mutagenic. Exceeds Minimum Criterion of 135.6 x 10^{-6}

[g]Not subcultured

[h]Not scored due to excessive toxicity

Table 44. Mutation Assay Results 4 hr +S9

Test Condition	Daily Cell Density/mL (x 10^5)		Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (x 10^{-6})[d]
	Day 1	Day 2								
Activation Controls[e]				AVG VC				AVG VC		
Vehicle Control	11.2	12.7	15.8		71	393	65.5		85.6	36.1
Vehicle Control	10.3	12.9	14.8		104	523	87.1		106.4	39.7
Vehicle Control	10.1	13.6	15.3	15.3	122	508	84.7	79.1	107.0	48.1
MCA 5 µg/mL	6.4	10.9	7.8		576	371	61.8		39.7	310.6[f]
MCA 10 µg/mL	5.8	9.1	5.9		548	323	53.8		26.1	339.2[f]
Test Article (µg/mL)			Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
50.0	11.3	10.3	84.7		108	632	133.1		112.7	34.2
100	10.8	11.1	87.2		75	630	132.6		115.7	23.9
200	11.5	11.2	93.7		84	499	105.1		98.4	33.7
250	10.1	12.4	91.1		71	509	107.4		97.8	27.8
275	9.1	10.8	71.5		98	600	126.4		90.4	32.7
300	5.3	14.0	54.0		82	434	91.5		49.4	37.7
325	3.2[g]	14.1	30.8		133	560	117.9		36.3	47.6
350	1.2[g]	7.3	15.9		84	417	87.8		14.0	40.3

[a]RSG = (Day 1 Count/3) x (Day 2 Count)/3 (or Day 1 Count if not subcultured)
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c]Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}
[e]Vehicle Control = 1% DMSO
Positive Control: MCA = Methylcholanthrene
[f]Mutagenic. Exceeds Minimum Criterion of 131.3 x 10^{-6}
[g]Not subcultured

To confirm the negative results, the study was repeated for 24 hr in the absence of S9 and again there was no significant increase in mutant frequency (see sponsor's table below).

Table 45. Mutation Assay Results 24 hr -S9

Test Condition	Daily Cell Density/mL (x 10^5)			Cumulative RSG[a]		Total Mutant Colonies	Total Viable Colonies	Cloning Efficiency[b]		Relative Growth (%)[c]	Mutant Frequency (x 10^{-6})[d]
	Day 1	Day 2	Day 3		AVG VC				AVG VC		
Nonactivation Controls[e]											
Vehicle Control	11.1	9.5	11.4	44.5		98	515	85.8		107.0	38.1
Vehicle Control	10.0	9.8	12.2	44.3		91	472	78.7		97.6	38.3
Vehicle Control	9.6	10.9	11.4	44.2	44.3	96	463	77.1	80.6	95.4	41.5
MMS 6.5 µg/mL	7.1	8.2	10.4	22.4		314	209	34.9		21.9	300.0[f]
MMS 9.0 µg/mL	7.9	9.3	10.1	27.5		315	214	35.6		27.4	294.9[f]
Test Article (µg/mL)				Relative to Vehicle Control (%)				Relative to Vehicle Control (%)			
12.5	10.0	10.1	12.0	101.3		110	525	108.6		109.9	42.0
25.0	10.9	10.0	11.2	102.0		88	443	91.6		93.5	39.9
50.0	11.5	8.2	10.4	81.9		79	446	92.3		75.7	35.2
75.0	8.7	6.2	11.3	50.9		63	520	107.7		54.8	24.3
100	5.8	7.1	11.8	40.6		88	528	109.3		44.4	33.5
125	5.1	4.6	10.6	20.8		104	440	91.0		18.9	47.1
150	4.6	6.6	8.7	22.1		88	332	68.6		15.1	53.3
175	2.6[g]	4.8	9.5	11.4		89	457	94.6		10.8	39.1

[a]RSG = [Treatment termination (Day 1) cell density/3] x [Day 2 cell density/3 or Day 1 density if not split back] x [Day 3 cell density/3 or Day 2 density if not split back]

[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100

[c]Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100

[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})

Decimal is moved to express the frequency in units of 10^{-6}

[e]Vehicle Control = 1% DMSO

Positive Control: MMS = Methyl methanesulfonate

[f]Mutagenic. Exceeds Minimum Criterion of 129.3 x 10^{-6}

[g]Not subcultured

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

A 3 Day Micronucleus Assay in Rats Administered BI 10773 XX by Oral Gavage (Study No. 06R086)

Study no:	06R086, U07-3543
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharmaceuticals 900 Ridgebury Road Ridgefield, CT 06877, USA
Date of study initiation:	16th May 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 10773 XX, BI10773XXA06 and 98.8%

Key Study Findings

Empagliflozin was negative in the in vivo micronucleus assay when using SD rats.

Methods

Doses in definitive study:	0, 100, 300, 1000 and 2000 mg/kg
Frequency of dosing:	Daily for 3 days
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.2% Natrosol (hydroxyethylcellulose)
Species/Strain:	Rat/SD
Number/Sex/Group:	5/sex/group
Satellite groups:	No
Basis of dose selection:	Not specified
Negative control:	0.2% Natrosol (hydroxyethylcellulose)
Positive control:	Cyclophosphamide (CP) 10 mg/kg

Study Validity

TK data confirms exposure. Positive result was induced by cyclophosphamide (CP). The dose formulations were within nominal concentrations.

Results

Treatment with empagliflozin for 3 days in the SD rat did not result in an increase in bone marrow micronucleated polychromatic erythrocytes (MN-PCE).

Table 46. Summary of Rat Micronucleus Bone Marrow Assessment (sponsor's table)

Micronucleus Summary

Test Article	Dose (mg/kg)	No. of Animals	Mean %PCE[a]	Mean %MN-PCE[a]
Vehicle	0	4M	60.8±4.2	0.10±0.00
		5F	63.3±1.9	0.08±0.03
BI 10773 XX	100	5M	NS[b]	NS
		5F	NS	NS
	300	5M	60.9±4.1	0.10±0.05
		5F	59.5±2.7	0.09±0.04
	1000	5M	60.1±4.5	0.07±0.03
		5F	59.7±4.6	0.10±0.04
	2000	5M	61.1±4.7	0.10±0.05
	10	5F	58.5±3.3	0.09±0.04
Cyclophosphamide		5M	47.1±5.6	0.81±0.22
		5F	47.1±4.6	0.83±0.14

a Mean ± Standard Deviation
b NS = Not scored

A 3 Day Micronucleus Assay in Rats Administered BI 10773 XX by Oral Gavage (Study No. 06R141)

Study no:	06R141, U07-3233
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharmaceuticals 900 Ridgebury Road Ridgefield, CT 06877, USA
Date of study initiation:	9th August 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 10773 XX, BI10773XXA06 and 98.8%

Key Study Findings

The sponsor conducted a second three day micronucleus study in the Wistar rat. Empagliflozin was negative in the in vivo micronucleus assay.

Methods

Doses in definitive study:	0, 100, 300, 1000 and 2000 mg/kg
Frequency of dosing:	Daily for 3 days
Route of administration:	Oral Gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.2% Natrosol
Species/Strain:	Rat/Wistar
Number/Sex/Group:	5/sex/group
Satellite groups:	None
Basis of dose selection:	Not secified
Negative control:	0.2% Natrosol (hydroxyethylcellulose)
Positive control:	Cyclophosphamide (CP) 10 mg/kg

Study Validity

TK data confirms exposure. Positive and negative controls produced the expected responses. The dose formulations were within nominal concentrations.

Results

Treatment with empagliflozin for 3 days in the Wistar rat did not result in an increase in bone marrow micronucleated polychromatic erythrocytes (MN-PCE).

Table 47. Summary of Rat Micronucleus Bone Marrow Assessment (sponsor's table)

Micronucleus Summary

Test Article	Dose (mg/kg)	No. of Animals	Mean %PCE[a]	Mean %MN-PCE[a]
Vehicle	0	4M	61.3±2.2	0.08±0.03
		5F	61.2±1.4	0.12±0.07
BI 10773 XX	300	5M	58.5±5.1	0.08±0.03
		5F	61.8±2.9	0.08±0.03
	1000	5M	62.3±3.4	0.11±0.02
		5F	58.8±5.6	0.09±0.02
	2000	5M	60.0±2.2	0.08±0.03
		5F	56.3±4.6	0.1±0.04
Cyclophosphamide	10	5M	47.1±6.8	0.73±0.17
		5F	45.7±1.1	0.64±0.14

a Mean ± Standard Deviation

2-Week Oral (Gastric Intubation) Range-Finding Toxicity Study in the Rat with BI 10773 (BIPI Study No. 05R214)

Study no:	05R214, U06-3381
Study report location:	EDR
Conducting laboratory and location:	Boehringer Ingelheim Pharmaceuticals 900 Ridgebury Road Ridgefield, CT 06877, USA
Date of study initiation:	June 12th 2006
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	BI 10773, TSA-05-006 and 99.1%

Key Study Findings

Treatment with empagliflozin for 2 weeks in the rat did not result in an increase in bone marrow micronucleated polychromatic erythrocytes (MN-PCE).

Methods

Doses in definitive study:	0, 30, 100, 300 and 500 mg/kg
Frequency of dosing:	Daily for 2 Weeks
Route of administration:	Oral Gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% Natrosol (hydroxyethylcellulose)
Species/Strain:	Rat/Wistar (Han)
Number/Sex/Group:	10/sex/group
Satellite groups:	None
Basis of dose selection:	None. This is the DRF study
Negative control:	0.5% Natrosol (hydroxyethylcellulose)
Positive control:	NA

Method

Blood was collected at the terminal necropsy from surviving animals. The immunochemical reagent anti-CD71-FITC differentially labels polychromatic erythrocytes (PCE) from normochromatic erythrocytes and propidium iodide detects micronucleated PCE (MN-PCE). Flow cytometric analysis was conducted for 20, 000 polychromatic erythrocytes (PCE).

Study Validity

TK data confirms exposure.

Results

The polychromatic erythrocytes (PCE) frequency was similar in control and empagliflozin treated rats. The frequency of MN-PCE was not statistically significantly

increased. **Reviewer note**: The sponsor only provides a written description of the micronucleus data as a memo to the main study report.

7.4 Other Genetic Toxicity Studies

In the drug substance impurities (b) (4) were identified as follows (sponsor's table):

Table 48. Empagliflozin Drug Substance Impurities

	Impurity		
	(b) (4)		
Percentage Impurity in Toxicology Study Batch			
Toxicology Study and Batch Identifier	Batch (b) (4) 4 week rat toxicity study	Batch (b) (4) 13 week rat toxicity study	Batch (b) (4) 13 week rat toxicity study (b) (4)

Impurities (b) (4) were qualified by their use in 4 and 13 week pivotal nonclinical studies in the rat and also by evaluation in an Ames assay and an in vitro micronucleus study in CHO cells. In the genetic toxicology studies impurities (b) (4) were found not to be mutagenic or clastogenic.

8 Carcinogenicity

104-Week Oral Carcinogenicity Study and Toxicokinetic Study With BI 10773 in Rats

Study no.:	09r001 Document No. U12-3580-01
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 9th 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 10773, Empagliflozin, Lot # 15 and purity 99.6%
CAC concurrence:	Yes

Key Study Findings

Statistically Significant Neoplastic Findings

- Whole body/cavity hemangioma and testicular Leydig cell tumors were independently identified by FDA biostatistics staff using trend analysis or pair-wise analysis and showed statistical significance ($p<0.05$). Incidence of these tumors was increased in the mid or high dose males when compared to the vehicle control males. Both tumor types were considered related to empagliflozin.
- Cervical, endometrial benign polyp (female only), thyroid adenoma (males only) and thyroid adenoma and carcinoma combined (males only) were identified as statistically significant ($p < 0.05$) by FDA biostatistics staff using pair-wise analysis in the 100 mg/kg females and the 300 mg/kg males, respectively. These were considered unrelated to drug treatment and lacked statistical significance by trend.

Non-Neoplastic Findings:

- Empagliflozin at 100, 300 and 700 mg/kg had minimal impact on survival. Slightly higher survival in the empagliflozin-treated animals may be due to treatment-related reduced body weight and body weight gain. Increased survival had no apparent effect on tumor incidence.
- Mean body weight was dose-dependently reduced in all empagliflozin treated females (up to 32%) and males (up to 42%).
- Exposure margins achieved were 17-21x, 26-45x and 42-72x in the low, mid and high dose males and females, respectively.
- Extensive kidney cortical tubule dilatation was observed in all empagliflozin-treated animals particularly the males.
- Mineralization was observed in the kidney tubules, heart, aorta and tongue of the empagliflozin-treated males and females and for the mesenteric lymph node and mandibular salivary gland in the 700 mg/kg males.
- Liver sinusoidal cell vacuolation and pancreatic acinar cell depletion was noted in all empagliflozin-treated animals.
- Bone accretion was observed in the 300 and 700 mg/kg males.
- In the femur a high incidence of residual cartilage of the diaphysis was observed in all empagliflozin-treated animals particularly at 300- and 700-mg/kg.

Maximum Clinical Exposure: 25mg/day, 4740 nM.hr. The high dose tested in this study achieved exposures of 42-fold and 72-fold the clinical exposure in males and females, respectively.

Adequacy of Carcinogenicity Study

The final study report of a GLP-compliant standard two year oral gavage carcinogenicity study in the Wistar (Han) rat was reviewed and the results were discussed at a meeting of the Executive Carcinogenicity Assessment Committee (ECAC). The ECAC and the Division considers the rat study an adequate assessment of carcinogenic potential because empagliflozin reached a ≥25x exposure margin in both males and females and

showed the high dose to be at the MTD due to reduced body weight compared to the controls.

Appropriateness of Test Models

The sponsor chose empagliflozin doses of 0, 0, 100, 300 and 700 mg/kg/day based on the recommendations of the ECAC. Overall treatment was well tolerated and the results showed no dose-limiting toxicity up to the highest dose tested. Exposure at the high dose (700 mg/kg) provided approximately 42x and 72x the maximum recommended human dose (MRHD) in males and females, respectively, based on total exposure ($AUC_{0\text{-}24h}$)

Evaluation of Tumor Findings

Methods

Doses:	0, 0, 100, 300 and 700 mg/kg
Frequency of dosing:	Once Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	C1: 0.5% hydroxyethylcellulose in water; C2: 0.5% hydroxyethylcellulose in water
Basis of dose selection:	Dose selection was done with ECAC concurrence
Species/Strain:	Rat/Wistar (Han), from (b) (4)
Number/Sex/Group:	50/sex/group
Age:	46-54 days
Animal housing:	The animals were individually housed in suspended, stainless steel, wire-mesh type cages
Paradigm for dietary restriction:	Food and water were provided *ad libitum*
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	TK; 12/sex/in treatment group and 6/sex in one control group dosed for 6 months
Deviation from study protocol:	None that affected study outcome

Observations and Results

Mortality

One 700 mg/kg male and female were euthanized moribund at day 7 and 5, respectively. Clinical signs for these animals included: hunched, swollen midline ventral abdomen, rough haircoat and non-formed feces. The study veterinarian also concluded that the animals were dehydrated.

Males and females that survived 104-weeks of treatment were necropsied at week 105. Survival rates were, in general, similar in the control, and treatment groups for both males and females. At termination the survival rates were 64, 70, 78 and 66% in males and 57, 64, 68 and 62% in females in 0 (groups 1 and 2 combined), 100, 300 and 700 mg/kg-treated groups, respectively. The summary of survivorship is shown in the sponsor's figures below.

Figure 14. Adjusted Survival (%): Males

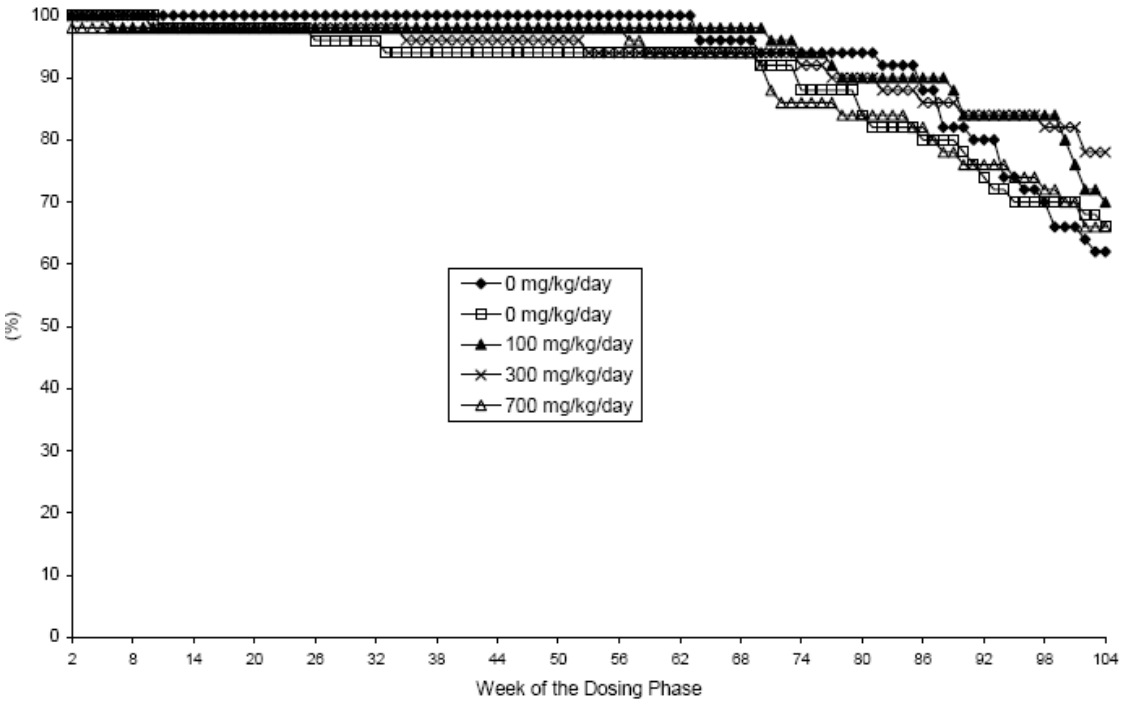
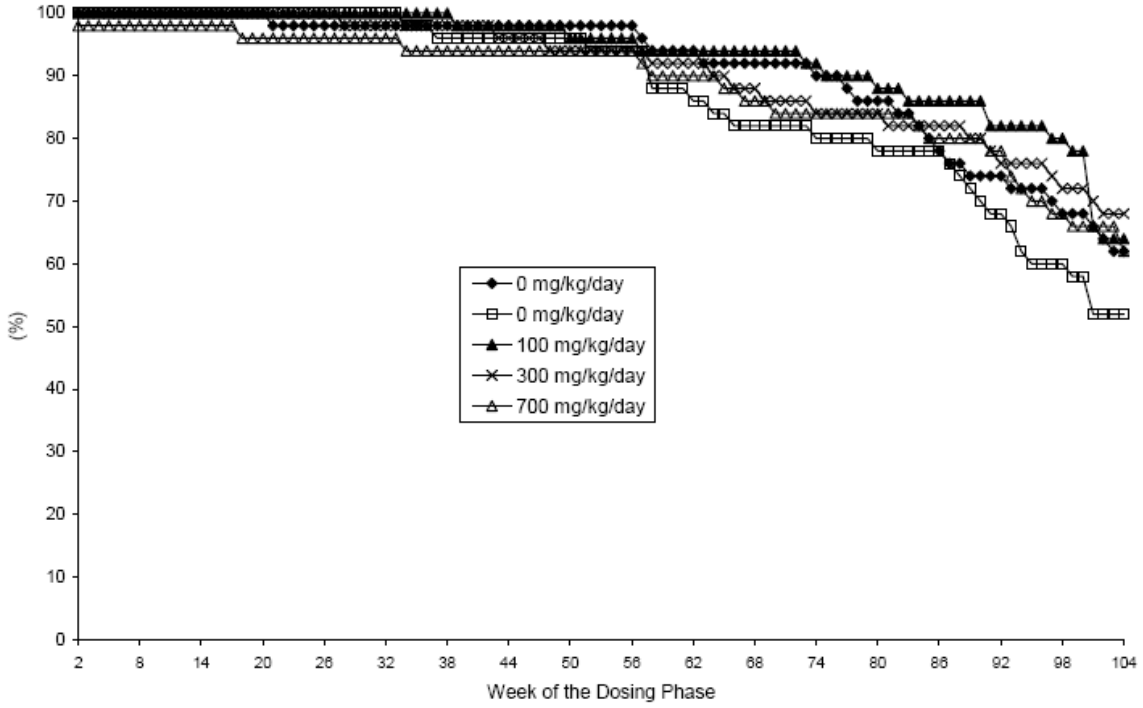


Figure 15. Adjusted Survival (%): Females



Pituitary neoplasm was the most common cause of neoplastic death in male and female rats including vehicle-treated rats. In females alone, the most common cause of death was mammary tumors (see sponsor's table below). In females the most common non-neoplastic cause of death was inflammation/obstruction of the urogenital tract at ≥ 300 mg/kg (see sponsor's table below). The microscopic findings associated with this finding varied and included pyelonephritis, uterine inflammation and/or uterine dilatation.

Table 49. Most Frequent Cause of Death

Most Frequent Causes of Death

Sex	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose Level (mg/kg/day)	0	0	100	300	700	0	0	100	300	700
Number Examined	50	50	50	50	50	50	50	50	50	50
Scheduled Euthanasia	31	33	35	39	32	31	26	32	33	31
Neoplasm, Pituitary	10	5	7	3	7	9	12	9	8	6
Undetermined	2	2	2	0	3	1	0	1	1	3
Inflammation/Obstruction, Urogenital Tract	0	1	0	1	1	0	0	0	2	6
Neoplasm, Mammary	0	0	0	0	0	1	6	1	2	0

Clinical Signs

The incidence of hunched, thin, rough haircoat, yellow haircoat (perineal area) and non-formed feces were increased in the 700 mg/kg animals and occurred primarily in weeks 1-2, except for clinical signs of rough haircoat and thin which also occurred throughout the remainder of the treatment phase. The high incidence (in some cases) and early

occurrence show an initial lack of tolerability of empagliflozin at 700 mg/kg and correlates with reduced body weight/body weight gain observed at 700 mg/kg.

The incidence of swollen, midline ventral abdomen was also increased in the 700 mg/kg males and females, respectively. In general, occurrences of this observation began for some 700 mg/kg animals at week 1-2 until week 16, and for other individual animals also at weeks 24, 28, 70 and 97, particularly in males. In addition, the incidence of swollen entire abdomen was increased in the 700 mg/kg females, but to a lesser extent than in the males and occurred either early (weeks 2-5) or late (weeks 56-69 or 90-104) in the treatment phase. Swollen abdomen and swollen midline ventral abdomen likely occurred due to off-target inhibition of SGLT1 which is primarily located in the intestine or is due to carbohydrate malabsorption.

Table 50. Clinical Signs - Male

Clinical signs	Male				
	0	0	100 mg/kg	300 mg/kg	700 mg/kg
Number of animals per group/Incidence	50	50	50	50	50
Hunched	3	1	6	3	14
Swollen midline ventral abdomen	0	0	0	2	44
Swollen entire abdomen	0	1	0	3	4
Thin	3	5	8	5	12
Feces, non-formed	0	0	3	1	45
Rough Haircoat	4	6	4	5	44
Yellow Haircoat, midline ventral abdomen	0	0	2	0	10
Yellow Haircoat, Perineal area	4	5	2	9	39

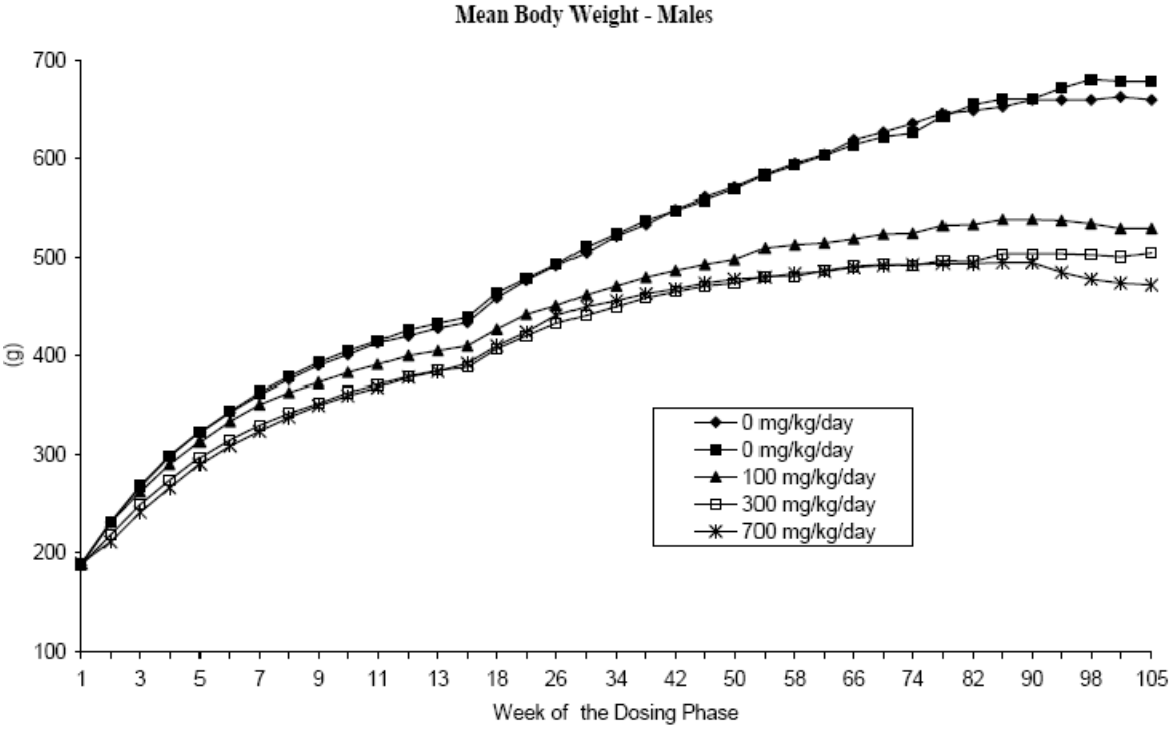
Table 51. Clinical Signs - Female

Clinical signs	Female				
	0	0	100 mg/kg	300 mg/kg	700 mg/kg
Number of animals per group/Incidence	50	50	50	50	50
Hunched	5	10	4	9	17
Swollen midline ventral abdomen	3	0	0	3	45
Swollen entire abdomen	0	1	1	4	20
Thin	11	8	10	10	12
Feces, non-formed	0	0	1	2	33
Rough Haircoat	11	13	7	6	35
Yellow Haircoat, midline ventral abdomen	0	2	0	1	13
Yellow Haircoat, Perineal area	3	8	1	4	23

Body Weights

The mean body weights were generally lower in the empagliflozin-treated males. At the end of treatment mean body weights were reduced 21%, 25% and 29% in the 100, 300 and 700 mg/kg-treated males, respectively, when using combined vehicle controls (see sponsor's figure below). Mean body weights were statistically significantly (ss) reduced in males in the 300 and 700 mg/kg groups beginning week 2 and in the 100 mg/kg group beginning week 5, respectively; and then throughout the remainder of the treatment phase for the mid and high dose males. In the males reduced mean body weight was dose dependently reduced at weeks 5-6, 8-11 and 86-105. Reduced mean body weight did not correlate with an increase in food consumption that was observed in the empagliflozin-treated males. **Reviewer note:** reduced body weight is likely due to caloric loss due to glucosuria, despite increased food consumption. Glucosuria is a known pharmacodynamic effect of SGLT2 inhibitors. Reduced body weight decreased spontaneous pituitary adenoma in the high dose females but not for high dose males. Reduced body weight also reduced spontaneous mammary fibroadenoma in the mid and high dose females.

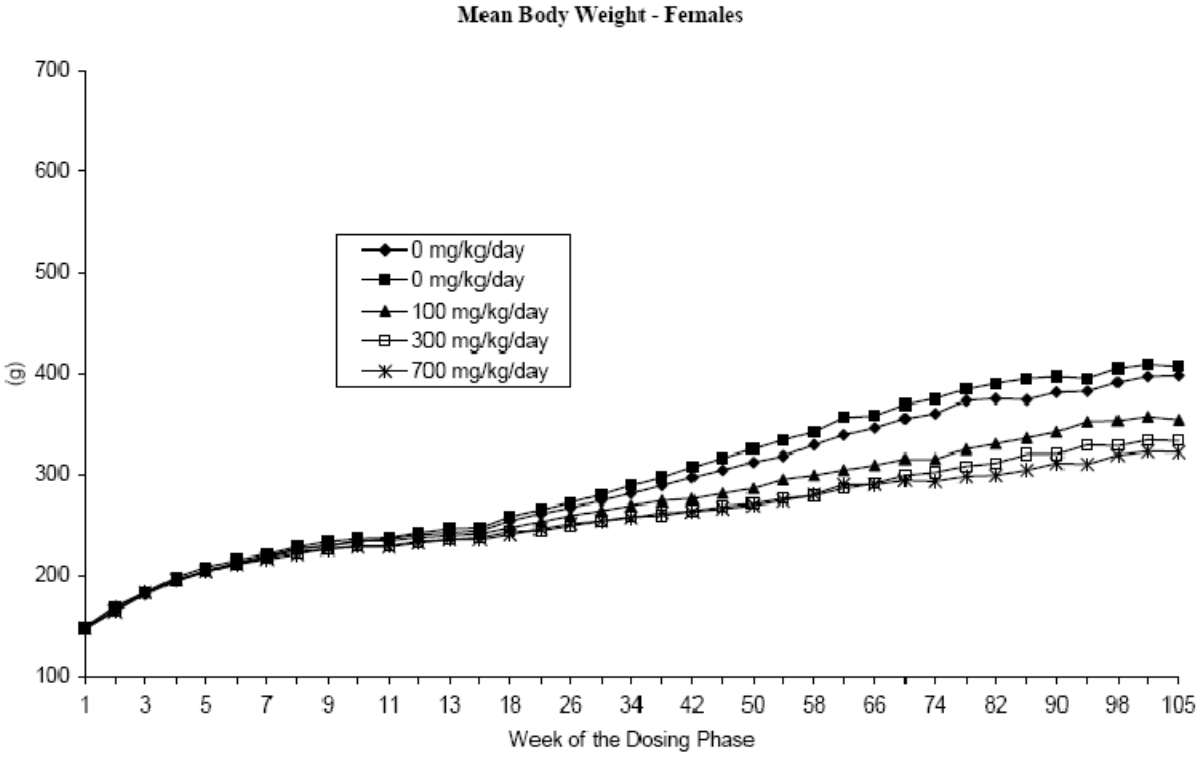
Figure 16. Mean Body Weight - Males



Mean body weight was also reduced in the empagliflozin-treated females. At the end of treatment mean body weights were reduced 12%, 17% and 20% in the 100, 300 and 700 mg/kg-treated females respectively (see sponsor's figure below). However, a ss reduction of mean body weight did not develop until week 11 in the 300 and 700 mg/kg-treated females and not until week 18 in the 100 mg/kg females, respectively. Mean body weight was ss reduced in the empagliflozin-treated females throughout the remainder of the treatment phase and dose-dependently reduced beginning week 70-

105. Reduced mean body weight did not correlate with an increase in food consumption observed in the empagliflozin-treated females.

Figure 17. Mean Body Weight - Females



Feed Consumption

Beginning at week 2 mean food consumption was statistically significantly (ss) increased in all empagliflozin treated animals until the study termination. Statistical significance was determined against combined vehicle groups 1 and 2 which both used 0.5% hydroxyethylcellulose in water. Food consumption was dose-dependently increased in males and females at several time points through out the treatment phase (see sponsor's figures below). Increased food consumption did not correlate with the reduced mean body weight/body weight gain in the empagliflozin-treated animals, likely due to caloric loss of glucose in the urine (not measured) which is a known pharmacodynamic effect for this drug class.

Figure 18. Food Consumption – Males

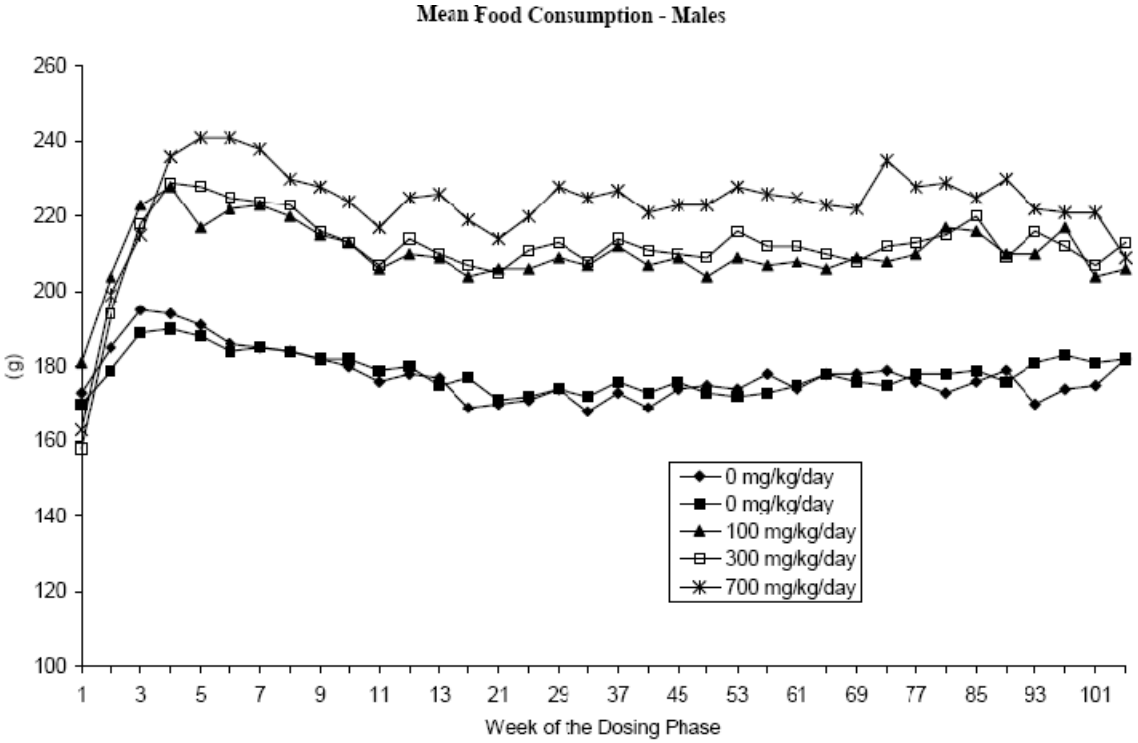
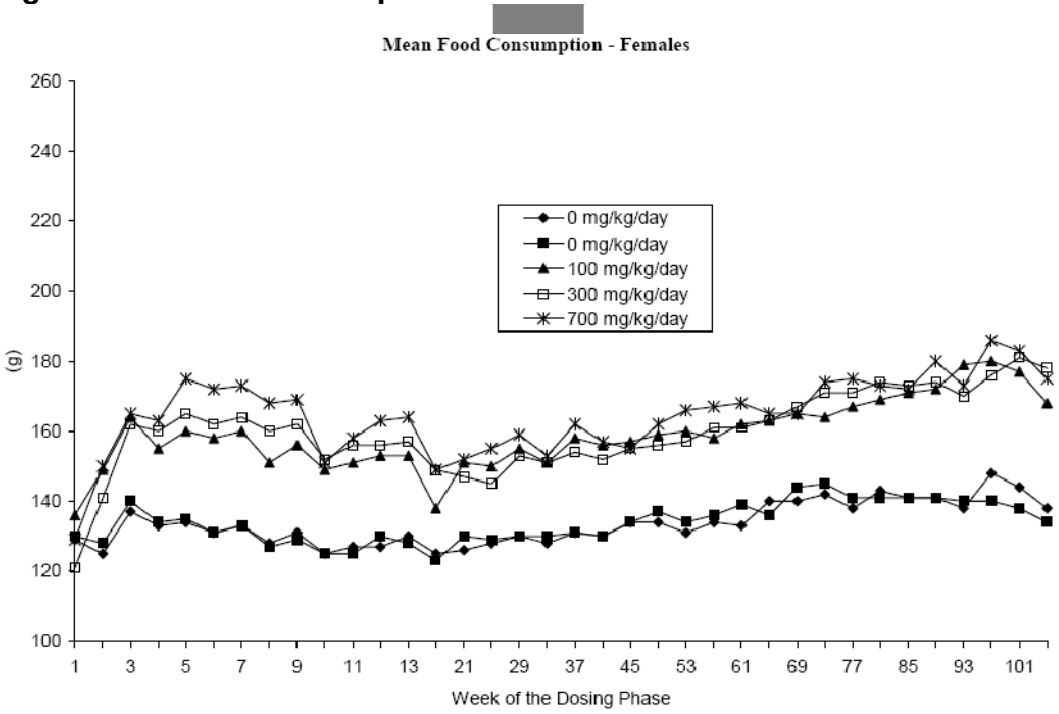


Figure 19. Food Consumption – Females



Gross Pathology

Scheduled Sacrifice

Discolored pituitary was noted with greater incidence in the empagliflozin-treated females and correlated microscopically with increased incidence of benign adenoma. There was an increased incidence of large kidneys in the low dose (100 mg/kg) males and this correlated microscopically with renal cortical tubule vacuolation, multifocal cortical tubule dilatation or tubule mineralization in the empagliflozin-treated males (see sponsor's table below).

There was also an increased incidence of discolored glandular stomach in the empagliflozin-treated animals, particularly in the empagliflozin-treated males and the 300 and 700 mg/kg females, respectively. The latter finding was without microscopic correlates in the males but corresponded with microscopic erosion/ulcer in the 300 and 700 mg/kg females, respectively. Discolored and large mesenteric lymph nodes (LN) were also observed in the 300 and 700 mg/kg males and correlated with an increased incidence of microscopic multicentric vascular neoplasm (hemangiomas) in the mesenteric LN. There was also an increased incidence of small seminal vesicle observed grossly in the 100 mg/kg males, which correlated microscopically with decreased secretion in the empagliflozin-treated males. Gross pathology also revealed increased incidence of discolored testes in the 300 and 700 mg/kg males which correlated microscopically with an increased incidence of benign interstitial (Leydig) cell tumors (see sponsor's table below).

Table 52. Scheduled Sacrifices Gross Pathology

Summary of Macroscopic Observations - Scheduled Sacrifice

	-- Males --					-- Females --				
Group:	1	2	3	4	5	1	2	3	4	5
Number in group:	31	33	35	39	32	31	26	32	33	31
Pituitary										
Discolored	3	3	0	4	2	4	4	8	9	8
Large	2	0	1	0	0	2	2	2	2	0
Mass	6	2	1	2	4	8	5	9	8	7
Raised Area	1	0	0	0	1	1	2	0	1	0
Total:	12	5	2	6	7	15	13	19	20	15
Kidney										
Contains Fluid	0	0	0	0	0	0	0	1	0	0
Cyst	1	0	0	1	1	1	0	0	1	0
Discolored	0	1	0	2	0	1	0	1	0	0
Granular Material	1	0	0	0	0	0	0	0	0	0
Large	3	2	7	3	1	0	0	0	1	1
Mass	0	0	0	0	0	0	0	0	1	0
Raised Area	0	0	1	0	0	0	0	0	0	0
Rough Surface	0	2	0	0	0	0	0	0	0	0
Total:	5	5	8	6	2	2	0	2	3	1
Stomach, Gl										
Discolored	7	5	16	18	16	3	3	5	9	9
LN, Mesenteric										
Discolored	1	1	1	4	5	0	2	0	1	0
Large	2	1	0	2	5	1	1	0	0	0
Total:	3	2	1	6	10	1	3	0	1	0
Seminal Vesicle										
Small	2	1	5	3	2	0	0	0	0	0
Total:	2	1	5	3	2	0	0	0	0	0

Table 53. Scheduled Sacrifices Gross Pathology - Continued

Summary of Macroscopic Observations - Scheduled Sacrifice

	-- Males --					-- Females --				
Group:	1	2	3	4	5	1	2	3	4	5
Number in group:	31	33	35	39	32	31	26	32	33	31
Testis										
Contains Fluid	2	1	0	2	1	0	0	0	0	0
Discolored	1	0	2	6	4	0	0	0	0	0
Large	2	1	1	2	2	0	0	0	0	0
Small	1	0	2	0	3	0	0	0	0	0
Soft	4	1	2	2	6	0	0	0	0	0
Total:	10	3	7	12	16	0	0	0	0	0

Unscheduled Deaths

There was an increased incidence of discolored kidneys observed macroscopically in the 300 and 700 mg/kg-treated males. In addition, an increased incidence of large kidneys was noted in the 700 mg/kg-treated males (see sponsor's table below). The renal gross pathology observations correlated microscopically with multifocal cortical tubule dilatation and tubule mineralization.

An increased incidence of discolored and large mesenteric lymph nodes was observed in the 300 and 700 mg/kg males, respectively, and correlated microscopically with an increased incidence of multicentric hematopoietic neoplasm and multicentric vascular neoplasm (hemangiomas) in the 300 and 700 mg/kg males, respectively. The incidence of small seminal vesicles were increased in the 700 mg/kg males and correlated microscopically with decreased secretion. The incidence of distended uterus was increased in the 700 mg/kg females and correlated microscopically with dilatation (see sponsor's table below).

Table 54. Unscheduled Sacrifices Gross Pathology

Summary of Macroscopic Observations
Unscheduled Sacrifices and Deaths

	-- Males --					-- Females --				
Group:	1	2	3	4	5	1	2	3	4	5
Number in group:	10	17	10	11	10	10	24	10	17	10
Kidney										
Abnormal Shape	0	0	0	0	0	0	0	1	0	0
Calculus	0	1	0	0	0	0	0	1	0	0
Contains Fluid	0	0	1	0	1	0	0	0	1	0
Cyst	0	0	0	1	0	0	0	0	0	0
Discolored	1	0	0	3	4	1	0	0	0	1
Large	1	1	1	1	4	1	1	2	1	1
Mass	0	1	0	0	0	0	0	0	0	2
Raised Area	0	0	0	0	0	0	0	0	0	1
Confirmed mass	0	0	0	0	0	1	0	0	0	0
Total:	2	3	2	5	9	3	1	4	2	5
LN, Mesenteric										
Cyst	0	0	0	0	0	0	0	0	0	1
Discolored	0	0	1	3	1	1	0	1	0	0
Large	0	0	1	2	2	0	0	1	1	0
Mass	0	0	1	0	0	0	0	0	1	0
Not Identified	0	0	1	0	0	0	0	0	0	0
Total:	0	0	4	5	3	1	0	2	2	1
Seminal Vesicle										
Discolored	0	0	0	1	0	0	0	0	0	0
Gelatinous	0	0	0	0	2	0	0	0	0	0
Small	4	2	1	4	9	0	0	0	0	0
Total:	4	2	1	5	11	0	0	0	0	0
Uterus										
Cyst	0	0	0	0	0	2	2	2	0	2
Distended	0	0	0	0	0	0	0	0	1	3
Intussusception	0	0	0	0	0	0	0	1	0	0
Large	0	0	0	0	0	1	0	0	0	1
Mass	0	0	0	0	0	0	0	2	0	1
Thickened	0	0	0	0	0	1	0	0	0	0
Total:	0	0	0	0	0	4	2	5	1	7

Total Unscheduled and Scheduled Sacrifices

The incidence of discolored pituitary was increased in the 300 mg/kg males and the empagliflozin-treated females and this incidence is from animals terminated at the scheduled sacrifice. The incidence of discolored kidney was also increased in the 300 mg/kg males and correlates microscopically with multifocal cortical tubule dilatation. Discolored glandular (Gl) stomach was increased in the 300 and 700 mg/kg treated females and all of the empagliflozin-treated males and correlated microscopically minimal congestion/hemorrhage or dilatation or was without microscopic correlates.

Large and discolored mesenteric lymph node (LN) was increased in the 300 and 700 mg/kg males and correlated microscopically with dilatation, multicentric hematopoietic neoplasm or multicentric vascular neoplasm, respectively.

The incidence of small seminal vesicles increased in the 700 mg/kg males and correlated with the microscopic incidence of decreased secretion. In addition, the incidence of discolored testes were increased in the 300 and 700 mg/kg males and correlated microscopically with benign interstitial (Leydig) cell tumor. Small testes was increased in the 700 mg/kg males and correlated microscopically with atrophy/degeneration.

Table 55. Gross Pathology Scheduled and Unscheduled Sacrifices

Summary of Macroscopic Observations - All Animals

	-- Males --					-- Females --				
Group:	1	2	3	4	5	1	2	3	4	5
Number in group:	50	50	50	50	50	50	50	50	50	50
Pituitary										
Cyst	0	1	0	0	0	0	0	0	0	0
Discolored	3	4	0	7	2	5	5	9	9	8
Large	2	0	1	0	0	2	2	2	2	0
Mass	18	8	8	5	12	19	19	18	18	14
Raised Area	1	0	0	0	1	1	2	0	1	1
Total:	24	13	9	12	15	27	28	28	30	23
Kidney										
Abnormal Shape	0	0	0	0	0	0	0	1	0	0
Calculus	0	1	0	0	0	0	0	1	0	0
Contains Fluid	0	0	1	0	1	0	0	1	1	0
Cyst	1	0	0	2	1	1	0	0	1	0
Discolored	1	1	0	5	4	2	0	1	0	1
Granular Material	1	0	0	0	0	0	0	0	0	0
Large	4	3	8	4	5	1	1	2	2	2
Mass	0	1	0	0	0	0	0	0	1	2
Raised Area	0	0	1	0	0	0	0	0	0	1
Rough Surface	0	2	0	0	0	0	0	0	0	0
Confirmed mass	0	0	0	0	0	1	0	0	0	0
Total:	7	8	10	11	11	5	1	6	5	6
Stomach, Gl										
Depressed Area	1	0	0	0	0	0	0	0	0	0
Discolored	9	5	18	21	19	5	4	6	14	11
Distended	0	0	1	0	0	0	0	0	0	0
Foreign Material	0	1	1	0	0	0	0	0	0	1
Mass	0	0	0	0	1	0	0	0	1	0
Raised Area	0	2	0	1	1	0	0	0	0	0
Rough Surface	0	0	0	0	0	0	0	0	0	1
Ulceration	0	0	0	1	1	0	0	1	0	0
Total:	10	8	20	23	22	5	4	7	15	13
LN, Mesenteric										
Cyst	0	0	0	0	0	0	0	0	0	1
Discolored	1	1	2	7	6	1	2	1	1	0
Large	2	1	1	4	7	1	1	1	1	0
Mass	0	0	1	0	0	0	0	0	1	0
Not Identified	0	0	1	0	0	0	0	0	0	0
Total:	3	2	5	11	13	2	3	2	3	1

Testis										
Contains Fluid	2	1	0	2	1	0	0	0	0	0
Discolored	1	0	2	7	6	0	0	0	0	0
Large	2	1	1	3	2	0	0	0	0	0
Small	2	1	2	1	5	0	0	0	0	0
Soft	9	1	5	5	10	0	0	0	0	0
Total:	16	4	10	18	24	0	0	0	0	0
Epididymis										
Discolored	0	0	0	1	0	0	0	0	0	0
Large	0	0	1	0	0	0	0	0	0	0
Mass	1	3	1	1	1	0	0	0	0	0
Small	0	0	1	2	4	0	0	0	0	0
Total:	1	3	3	4	5	0	0	0	0	0

There were also no apparent drug-related increases in palpable masses.

Histopathology

Peer Review: Yes

Neoplastic
An increased incidence of benign vascular tumors (hemangiomas) was observed with 3, 0, 2, 5 and 9 hemangiomas in vehicle control 1, vehicle control 2, 100, 300 and 700 mg/kg males, respectively (see sponsor's table below). This finding correlated with an increased incidence of large (most) and discolored mesenteric lymph nodes observed grossly in the same group. Per the Biostatistical reviewer (Dr. Min) hemangiomas were statistically significant (ss) for trend analysis with vehicle control 2 (p = 0.001) (data shown in Appendix B) and when both vehicle control 1 and 2 were combined (p = 0.001) (see table 57 below). Using pair-wise analysis, hemangiomas were not ss for vehicle control 1, but showed statistical significance for vehicle control 2 against the 700 mg/kg males (p = 0.001) (see Appendix B) and also for the combined vehicle 1 and 2 against the 700 mg/kg males (p = 0.002), respectively (see table 57 below). Hemangiosarcomas were absent in males and at a very low incidence in females.

Table 56. Neoplastic Findings in Male and Female Rats

Sex	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose Level (mg/kg/day)	0	0	100	300	700	0	0	100	300	700
Mesenteric Lymph Node										
Number Examined	50	50	50	50	50	50	48	50	50	50
Hemangioma	3	0	1	4	9	0	1	0	0	0
Body Whole/Cavity										
Number Examined	50	50	50	50	50	50	50	50	50	50
Hemangioma	3	0	2	5	9	0	1	0	0	0
Hemangiosarcoma	0	0	0	0	0	1	1	0	0	0
Testis										
Number Examined	50	50	50	50	50	NA	NA	NA	NA	NA
Interstitial Cell Tumor	2	0	4	7	6	NA	NA	NA	NA	NA

NA = Not applicable.

Whole body/cavity lymphosarcoma was also noted 0, 0, 0, 5 and 0 in vehicle control 1, vehicle control 2, 100, 300 and 700 mg/kg males, respectively. Per the Biostatistics reviewer whole body/cavity lymphosarcoma in the 300 mg/kg males was statistically significant using pair wise analysis when using vehicle control 1 ($p = 0.033$), vehicle control 2 ($p = 0.037$) (see Appendix B) and also when vehicle control 1 and 2 were combined ($p = 0.005$) but not by trend analysis (see table 57 below). The absence of this tumor type at the high dose suggests that the increase at the mid dose is incidental to treatment.

Benign interstitial (Leydig) cell tumors of the testis were increased with 2, 4, 7 and 6 hemangiomas in the combined vehicle control, 100, 300 and 700 mg/kg males, respectively (see table 56 above). Per the Biostatistics reviewer (Dr. Min) Leydig cell tumors were statistically significant in a pair-wise analysis for vehicle control 2 against the 300 mg/kg males ($p = 0.008$) and the 700 mg/kg males ($p = 0.0014$) (see Appendix B) and a pair-wise analysis for combined vehicle 1 and 2 against the 300 mg/kg ($p = 0.008$) males (see table 57 below), respectively.

The incidence of spontaneous Leydig cell tumors in Wistar rats ranges from 4-7% (Bomhard and Rinke, 1994 and Walsh and Poteracki, 1994), but has also been found to as high as 14% (Nolte et.al., 2011). However, the incidence of Leydig cell tumors in humans is a rare event (0.00004%) (Gilliland and Key, 1995) and unlikely to be encountered in humans due to the physiological differences between the rat and human for this tumor (Cook et.al., 1999).

In the cervix, endometrial stromal benign polyp was present at 0, 0, 3, 0 and 1 in vehicle control 1, vehicle control 2, 100, 300 and 700 mg/kg females, respectively. Per the Biostatistics reviewer (Dr. Min) the cervical endometrial stromal benign polyp was statistically significant using pair-wise analysis when vehicle control 1 and 2 were combined and compared against the 100 mg/kg females ($p = 0.044$) (see table 57 below). Historical control data were not submitted.

Bomhard E, Rinke M: Exp. Toxicol. Pathol.: 46(1): 17-29 (1994)
Walsh KM, Poteracki J: Fundam. Appl. Toxicol.: 22(1): 65-72 (1994)
Nolte T, et.al.,: Exp Toxicol Path: 63(7): 645-656 (2011).
Gillilan FD, Key CR: Cancer: 75(1): 295-315 (1995)
Cook JC, et.al.,: Crit. Rev. Toxicol.: 29(2): 169-261 (1999).

Table 57. Rat Tumor Statistical Analysis

Summary of tumors with any significant difference from controls †										
Tissue/Tumor	Sex	Empagliflozin (mg/kg/day)					Statistics (p-value) [a]			
		0 (vehicle 1)	0 (vehicle 2)	100	300	700	Trend	Pair-wise Low-dose	Pair-wise Mid-dose	Pair-wise High-dose
Exposure Multiples		Males		17x	26x	42x				
		Females		21x	45x	72x				
Whole body/cavity: Hemangioma	M	3	0	2	5	9	0.001	nss	nss	0.002
Whole body/cavity: Lymphosarcoma	M	0	0	0	5	0	nss	nss	0.005	nss
Testis: Leydig cell tumor	M	2	0	4	7	6	nss	nss	0.008	nss
Cervix: Endometrial Benign Polyp	F	0	0	3	0	1	nss	0.044	nss	nss

† Statistical analyses summarized from FDA statistics review (Dr. Min), [a] Statistical analysis with vehicle 1 and vehicle 2 combined, F = female, M = male, nss = not statistically significant.

Non Neoplastic

Kidney

A dose-dependent minimal to moderate multifocal dilatation of the cortical tubules with increasing incidence, severity and dose-dependence was observed in the empagliflozin-treated males and in the mid (300 mg/kg) and high dose (700 mg/kg) females, respectively. According to the study pathologist this tubular dilatation differs from the tubular dilatation that arises due to chronic progressive nephropathy. Minimal focal/multifocal vacuolation of the cortical tubule was also observed in the empagliflozin-treated males with incidence in the following order low > mid > high dose males (see sponsor's table below). The study pathologist considered the tubular dilatation and vacuolation to be related to the empagliflozin treatment.

Minimal to slight kidney tubule mineralization was dose-dependently increased in the empagliflozin-treated males. The incidence of minimal to slight kidney tubule mineralization was also increased in the empagliflozin-treated females but was not dose-related. In contrast, the incidence of minimal to moderate mineralization of the pelvis was decreased in the empagliflozin-treated males and females, respectively (see sponsor's table below).

Table 58 Non-neoplastic Findings: Kidney

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose Level (mg/kg/day)	0	0	100	300	700	0	0	100	300	700
Kidney										
Number Examined	50	50	50	50	50	50	50	50	50	50
Dilatation, Cortical Tubules, Multifocal										
Minimal	1	4	27	34	24	4	6	8	16	19
Slight	0	0	1	6	19	0	1	1	0	8
Moderate	0	0	0	0	2	0	0	0	0	1
TOTAL	1	4	28	40	45	4	7	9	16	28
Vacuolation, Cortical Tubules, Focal/Multifocal										
Minimal	0	2	18	13	6	1	2	0	0	0
TOTAL	0	2	18	13	6	1	2	0	0	0
Mineralization, Tubule(s)										
Minimal	13	9	30	31	26	28	25	38	38	44
Slight	0	0	2	14	21	0	1	1	1	2
TOTAL	13	9	32	45	47	28	26	39	39	46
Mineralization, Pelvis										
Minimal	9	11	0	0	2	22	18	9	2	5
Slight	2	2	1	0	2	11	11	3	2	2
Moderate	1	0	0	1	0	0	0	0	0	0
TOTAL	12	13	1	1	4	33	29	12	4	7

Numbers in bold indicate BI 10773-related findings.

Mesenteric Lymph Node

In addition to the increased mesenteric lymph node hemangioma formation, non-neoplastic microscopic finding were also noted in the mesenteric lymph node. This included a treatment-related (minimal to slight) increase in sinus histiocytes, pigmented macrophages and mast cells particularly in the males and/or the 700 mg/kg females that was usually non-dose dependent (see sponsor's table below). Sinus erythrocytes were also non-dose dependently increased in the empagliflozin-treated males (see sponsor's table below). The increased incidence and severity (moderate) of the sinus erythrocytes in the 300 and 700 mg/kg males suggests chronicity of toxic insult and/or a lymph node draining a site of hemorrhage, the latter which could also be an artifact of necropsy (Elmore, 2006).

Table 59. Non-neoplastic Findings: Mesenteric Lymph Node

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose Level (mg/kg/day)	0	0	100	300	700	0	0	100	300	700
Mesenteric Lymph Node										
Number Examined	50	50	50	50	50	50	48	50	50	50
Histiocytes, Sinus, Increased										
Minimal	6	7	13	11	13	4	8	12	13	17
Slight	1	0	1	1	2	2	0	5	4	7
Moderate	0	0	0	0	0	0	0	0	1	1
TOTAL	7	7	**14**	**12**	**15**	6	8	**17**	**18**	**25**
Pigment, Macrophages, Increased										
Minimal	4	5	18	12	18	19	14	14	12	32
Slight	3	2	3	3	6	2	1	3	3	2
Moderate	0	0	0	0	1	0	0	0	1	1
TOTAL	7	7	**21**	**15**	**25**	21	15	17	16	**35**
Mast Cells, Increased										
Minimal	6	7	12	10	15	8	9	11	11	11
Slight	4	1	5	8	4	1	2	4	5	11
Moderate	0	1	0	1	2	0	0	0	1	0
TOTAL	10	9	**17**	**19**	**21**	9	11	15	17	**22**
Erythrocytes, Sinus										
Minimal	11	11	12	9	8	9	6	7	8	8
Slight	5	3	5	5	5	2	2	3	2	3
Moderate	2	1	3	6	8	0	2	1	2	1
TOTAL	18	15	20	**20**	**21**	11	10	11	12	12

Numbers in bold indicate BI 10773-related findings.

Elmore SA, Toxicol. Pathol.: 34(5): 425-454 (2006)

Urinary Bladder

Urinary bladder dilatation was slightly increased in the 700 mg/kg males (see sponsor's table below). All other urinary bladder non-neoplastic findings were unremarkable.

Table 60. Urinary Bladder Histopathology

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --					-- Animals Affected -- Females --				
Tissues With Diagnoses	Dosage group:	Ctls	2	3	4	5	Ctls	2	3	4	5
	No. in group:	50	50	50	50	50	50	50	50	50	50
Urinary Bladder ..... Number examined:		49	50	49	49	50	49	50	50	50	50
C-Hematopoietic Neoplasm, see Body, Whole for type											
	->	49	50	49	47	49	49	50	50	50	50
	^>	0	0	0	2	1	0	0	0	0	0
.....Total Incidence of Finding Observed:		0	0	0	2	1	0	0	0	0	0
Calculus											
	->	49	50	49	48	49	49	50	50	50	50
	P>	0	0	0	1	1	0	0	0	0	0
.....Total Incidence of Finding Observed:		0	0	0	1	1	0	0	0	0	0
Congestion/Hemorrhage											
	->	49	48	47	46	47	49	50	48	50	50
	1>	0	1	2	2	3	0	0	2	0	0
	3>	0	1	0	1	0	0	0	0	0	0
.....Total Incidence of Finding Observed:		0	2	2	3	3	0	0	2	0	0
Dilatation											
	->	49	49	49	49	47	48	50	49	50	50
	1>	0	0	0	0	1	1	0	0	0	0
	2>	0	0	0	0	2	0	0	0	0	0
	3>	0	1	0	0	0	0	0	1	0	0
.....Total Incidence of Finding Observed:		0	1	0	0	3	1	0	1	0	0
Urinary Bladder ..... Number examined:		49	50	49	49	50	49	50	50	50	50
Hyperplasia, Transitional Cell											
	->	48	49	45	48	47	49	50	46	49	49
	1>	0	0	1	0	0	0	0	1	1	1
	2>	1	0	0	1	1	0	0	2	0	0
	3>	0	1	2	0	2	0	0	1	0	0
	4>	0	0	1	0	0	0	0	0	0	0
.....Total Incidence of Finding Observed:		1	1	4	1	3	0	0	4	1	1
Infiltrate, Lymphocytes/Macrophages											
	->	47	47	47	49	49	49	46	46	49	48
	1>	2	3	2	0	1	0	4	3	1	2
	2>	0	0	0	0	0	0	0	1	0	0
.....Total Incidence of Finding Observed:		2	3	2	0	1	0	4	4	1	2
Inflammation											
	->	48	47	45	48	47	49	50	50	50	49
	1>	0	2	1	0	2	0	0	0	0	1
	2>	1	0	3	1	1	0	0	0	0	0
	3>	0	1	0	0	0	0	0	0	0	0
.....Total Incidence of Finding Observed:		1	3	4	1	3	0	0	0	0	1

Other Tissues

Additional noteworthy non-neoplastic findings are shown in the sponsor's table below. A dose-dependent increase in the incidence of minimal vacuolation of the hepatic sinusoidal cells was noted in the empagliflozin-treated animals (see sponsor's table below). Slight to moderate erosion of the glandular stomach (minimal to moderate) was observed in the 700 mg/kg males and the 300- and 700 mg/kg females. A dose-dependent increase in the incidence of minimal to slight granule depletion (minimal to slight) of the pancreatic acinar cells was observed in the empagliflozin-treated animals (see sponsor's table below). The latter pancreatic finding may be due to increased food consumption and reduced body weight/ body weight gain, observed in these animals.

Table 61. Non-neoplastic Findings: Mesenteric Live, Stomach, Pancreas, Bone and Seminal Vesicle

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose Level (mg/kg/day)	0	0	100	300	700	0	0	100	300	700
Liver										
Number Examined	50	50	50	50	50	50	50	50	50	50
Vacuolation, Sinusoidal Cells										
Minimal	1	1	11	20	25	1	1	16	19	21
TOTAL	1	1	**11**	**20**	**25**	1	1	**16**	**19**	**21**
Stomach, Glandular										
Number Examined	50	50	50	50	50	49	50	50	50	50
Erosion/Ulcer										
Minimal	4	2	2	1	5	1	0	0	2	3
Slight	0	1	0	1	6	1	0	1	6	5
Moderate	0	0	1	0	1	0	0	0	0	0
TOTAL	4	3	3	2	**12**	2	0	1	**8**	**8**
Pancreas										
Number Examined	50	50	50	50	49	50	50	50	50	50
Depletion, Acinar Cell										
Minimal	2	2	4	7	8	2	0	8	15	17
Slight	0	0	2	3	6	0	0	1	3	8
TOTAL	2	2	**6**	**10**	**14**	2	0	**9**	**18**	**25**
Bone, Femur										
Number Examined[a]	31	33	35	39	32	31	26	32	33	31
Residual Cartilage, Diaphysis, Increased										
Minimal	0	0	9	21	19	4	1	6	8	13
Slight	0	0	0	6	9	0	0	2	4	8
TOTAL	0	0	**9**	**27**	**28**	4	1	**8**	**12**	**21**
Bone, Sternum										
Number Examined[a]	31	33	35	39	32	31	26	32	33	31
Trabecular Bone, Increased										
Minimal	0	0	0	6	4	0	0	0	1	0
Slight	0	0	0	2	6	0	0	0	0	0
TOTAL	0	0	0	**8**	**10**	0	0	0	1	0
Seminal Vesicle										
Number Examined	49	50	50	50	50	NA	NA	NA	NA	NA
Decreased Secretion										
Minimal	3	6	8	6	9	NA	NA	NA	NA	NA
Slight	5	0	2	3	5	NA	NA	NA	NA	NA
TOTAL	8	6	10	9	**14**	NA	NA	NA	NA	NA

NA = Not applicable.
Numbers in bold indicate BI 10773-related findings..
a Scheduled euthanasia animals only

At the end of treatment, increased cartilage (minimal to slight) at the diaphysis of the femur was observed in the empagliflozin-treated animals but was very prominent in the 300 and 700 mg/kg males (see sponsor's table above). In addition, trabecular bone was increased (minimal to slight) in the sternum of the 300 and 700 mg/kg males that were examined at the end of treatment (see sponsor's table above). Bone accretion is

thought to occur as a result of increased calcium absorption in the GI tract, secondary to SGLT1 inhibition in the GI tract that results in reduced absorption of glucose and galactose, acidic fermentation of these sugars in the intestine, followed by increased absorption of dietary calcium. However, this finding was not associated with GI tract histopathology (cecal, duodenal etc.).

An increased incidence of decreased secretion (minimal to slight) of the seminal vesicle was observed in the 700 mg/kg group males and correlated with the gross pathology finding of small seminal vesicle in the same group. Soft tissue mineralization and vascular mineralization (minimal to moderate) was present in the heart, aorta, tongue, kidney, mesenteric lymph node and mandibular salivary gland in the males (predominantly) at ≥ 100 mg/kg and females at ≥ 300 mg/kg (sponsor's table below). Soft tissue mineralization and vascular mineralization appears to be a mechanism to deal with increased absorption of dietary calcium.

Table 62. Soft Tissue Mineralization of Empagliflozin-Treated Rats

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose Level (mg/kg/day)	0	0	100	300	700	0	0	100	300	700
Heart										
Number Examined	49	50	50	50	50	50	50	50	50	50
Mineralization, Vessel										
Minimal	0	0	7	11	10	1	0	1	4	6
Slight	0	0	0	4	13	0	0	0	0	0
Moderate	0	0	0	1	5	0	0	0	0	0
TOTAL	0	0	**7**	**16**	**28**	1	0	1	**4**	**6**
Aorta										
Number Examined	50	50	50	50	49	50	50	50	50	50
Mineralization										
Minimal	0	0	3	2	10	0	0	0	0	1
Slight	0	0	0	0	8	0	0	0	0	0
Moderate	0	0	0	0	2	0	0	0	0	0
TOTAL	0	0	**3**	**2**	**20**	0	0	0	0	**1**
Tongue										
Number Examined	50	50	50	50	50	50	50	50	50	50
Mineralization, Vessel										
Minimal	1	0	11	22	30	0	0	0	4	9
Slight	0	0	0	0	4	0	0	0	0	0
TOTAL	1	0	**11**	**22**	**34**	0	0	0	**4**	**9**
Kidney										
Number Examined	50	50	50	50	50	50	50	50	50	50
Mineralization, Vessel										
Minimal	0	0	2	6	13	0	0	0	0	2
Slight	0	0	0	0	1	0	0	0	0	0
TOTAL	0	0	**2**	**6**	**14**	0	0	0	0	**2**
Mesenteric Lymph Node										
Number Examined	50	50	50	50	50	50	48	50	50	50
Mineralization, Vessel										
Slight	0	0	0	0	5	0	0	0	0	0
Moderate	0	0	0	0	2	0	0	0	0	0
TOTAL	0	0	0	0	**7**	0	0	0	0	0
Mandibular Salivary Gland										
Number Examined	50	50	50	50	50	50	50	50	50	50
Mineralization, Vessel										
Minimal	0	0	0	0	3	0	0	0	0	0
TOTAL	0	0	0	0	**3**	0	0	0	0	0

Numbers in bold indicate BI 10773-related findings.

Toxicokinetics

In this 2-year rat carcinogenicity study the sponsor administered empagliflozin at 100, 300, 700 mg/kg. Blood was obtained from non-fasted satellite TK animals pre-dose and at day 87 and 178 of the treatment phase. T_{max} ranged from 1 to 4 hr and C_{max} and $AUC_{0\text{-}24h}$ were less than dose proportional in both males and females. Systemic exposure in the males was 0.6 to 0.9x lower compared to the female animals. Empagliflozin at 100 mg/kg appears to accumulate as the duration of exposure is increased. Sponsor's table below:

Table 63. Toxicokinetics

Toxicokinetic Parameters of BI 10773 after Oral Administration of BI 10773 XX during a 104-Week Oral Toxicity Study in Wistar Han Rats					
TK Parameter	Sex	Drug Day	BI 10773 XX (mg/kg/day)		
			100	300	700
C_{max} (nM)	Male	Day 1	4,540	10,300	30,100
		Day 87	8,620	9,760	21,600
		Day 178	10,000	20,500	23,700
	Female	Day 1	4,710	10,600	32,800
		Day 87	11,600	14,000	40,600
		Day 178	17,800	37,300	48,700
$AUC_{0\text{-}24}$ (nM·h)	Male	Day 1	44,500	127,000	265,000
		Day 87	58,700	90,100	221,000
		Day 178	79,800	122,000	197,000
	Female	Day 1	44,700	121,000	296,000
		Day 87	65,900	106,000	259,000
		Day 178	100,000	214,000	340,000
t_{max} (h)	Male	Day 1	1	2	4
		Day 87	2	2	4
		Day 178	2	4	4
	Female	Day 1	1	2	4
		Day 87	2	4	4
		Day 178	2	4	2

Dosing Solution Analysis

The sponsor states that the formulations were stable from study day 0 of preparation through to study day 18 under refrigerated conditions and under room temperature for 24h. However, this data was not provided in the study report. [The formulations were prepared weekly]. The concentration verification throughout the study were within 5% of the nominal concentration.

104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study with BI 10773 in Mice

Study no.:	09R002, 0286-422
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 2nd 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 10773, Lot #15, purity 99.6%
CAC concurrence:	Yes

Key Study Findings

Statistically Significant Neoplastic Findings

- Renal tubular adenoma and carcinoma combined were statistically significantly increased by trend analysis and pair-wise testing in the 1000 mg/kg males, accompanied by an even higher incidence of tubular atypical hyperplasia.
- Renal tubular hyperplasia (not atypical) was increased at all doses in empagliflozin-treated mice, particularly the males.

Non-Neoplastic Findings:

- Numerous findings indicate chronic tubule nephrotoxicity in males at 1000mg/kg, including single cell necrosis, karyomegaly, hypertrophy and atrophy, and cysts throughout the renal tubules. CPN was also exacerbated by empagliflozin.
- Less severe findings were observed at lower dose groups, notably tubular hypertrophy and hyperplasia, primarily in males.
- The incidence and severity ureter dilatation was exacerbated in the empagliflozin-treated males.
- The incidence of urinary bladder dilatation was exacerbated in the empagliflozin-treated males.
- Hepatic cytoplasmic vacuolation was increased in all animals at ≥ 300 mg/kg.
- Exposure margins achieved were 4-7x, 11-28x and 45-62x in the low, mid and high dose males and females, respectively.

Maximum Clinical Exposure: 25mg/day, 4740 nM.hr. The high dose tested in this study achieved exposures of 45-fold and 62-fold the clinical exposure in males and females, respectively.

Adequacy of Carcinogenicity Study

The final study report of a GLP-compliant standard two year oral gavage carcinogenicity study in the CD-1 mice was reviewed and the results were discussed at a meeting of the Executive Carcinogenicity Assessment Committee (ECAC). The ECAC and the

Division considers the mouse carcinogenicity study as an adequate tumor assessment because empagliflozin reached a ≥25x exposure margin in both males and females.

Appropriateness of Test Models

The sponsor chose empagliflozin doses of 0, 0, 100, 300 and 1000 mg/kg/day based on the recommendations of the ECAC. Overall treatment was well tolerated and the results showed no dose-limiting toxicity, except for excess mortality in the high dose male group which was terminated at week 97. As all high dose males and females were terminated at weeks 97 and 102, respectively, the high dose was considered adequate for tumor assessment and statistical evaluation. Exposure at the high dose (700 mg/kg) provided approximately 45x and 62x the maximum recommended human dose (MRHD) in males and females, respectively, based on total exposure ($AUC_{0\text{-}24h}$)

Evaluation of Tumor Findings

Methods

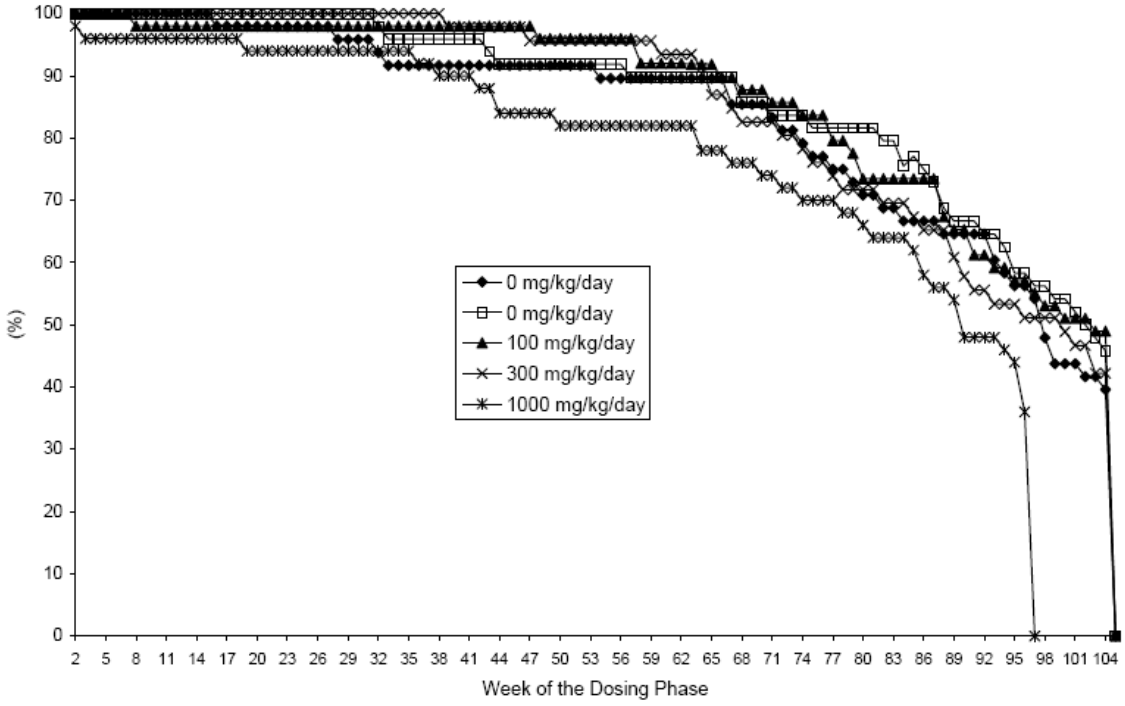
Doses:	M & F: 0, 0, 100, 300, and 1000 mg/kg
Frequency of dosing:	Once daily
Dose volume:	10 mL/Kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) hydroxyethylcellulose
Basis of dose selection:	Dose selection was done with ECAC concurrence
Species/Strain:	CD-1 Mice, from (b) (4)
Number/Sex/Group:	50/sex/group
Age:	38 to 46 days old
Animal housing:	The animals were individually housed in polycarbonate cages
Paradigm for dietary restriction:	Food and water were provided *ad libitum*
Dual control employed:	Yes
Interim sacrifice:	High dose males were terminated at week 97
Satellite groups:	TK: 25/sex/group dosed for 6 months
Deviation from study protocol:	None that affected study outcome

Observations and Results

Mortality

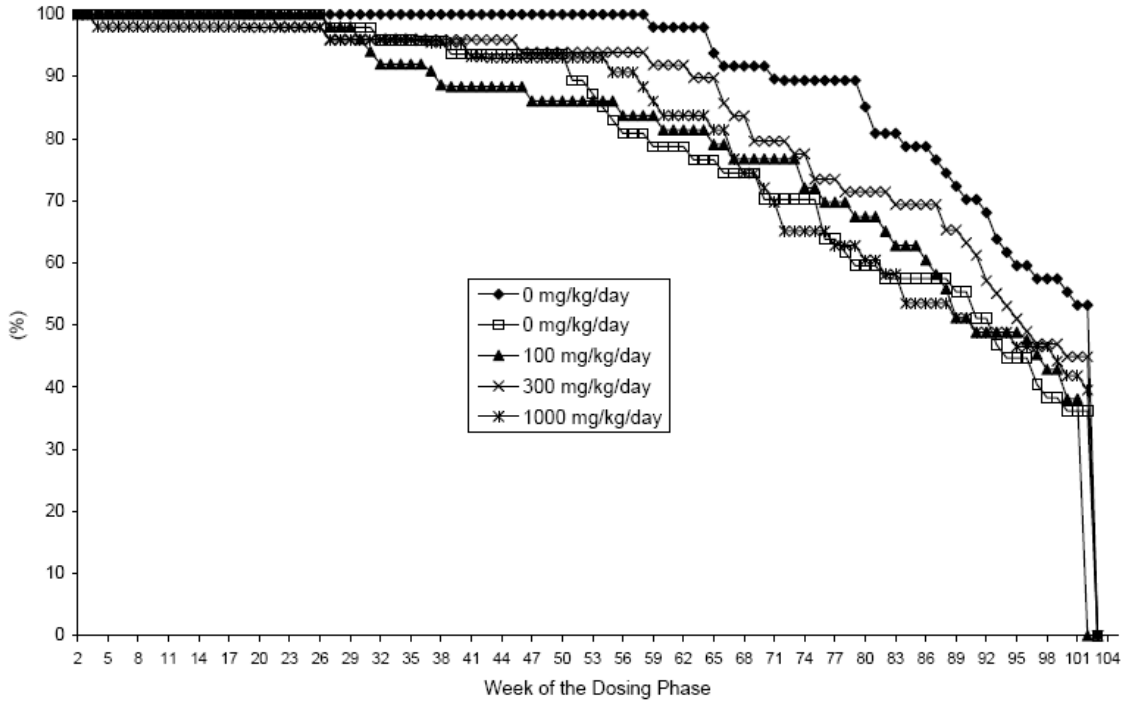
Decreased survival was observed in the 1000 mg/kg males that resulted in early termination for this group at week 97 (see sponsor's figure below). At study termination 16 males and 17 females were present in the high dose (HD) group. Although, a submission to the Division and ECAC was not made to request early termination of the HD male group, it is likely ECAC would have concurred if the number of survivors had reached 15 or less, prior to week 100 of the study. Increased mortality observed for the HD male group was due to urinary tract dilatation. Given that all HD males and females were terminated at week 97 and 102, respectively, the HD group is considered adequate for tumor assessment and statistical evaluation.

Figure 20. Adjusted Survival (%): Males



The sponsor states that survival was not affected in the BI 10773-treated females. However, vehicle control groups 1 and 2 appear to have slightly different distributions of survival times and are different from each other (see sponsor's figure below). Survival was unaffected in the empagliflozin-treated female groups (see sponsor's figure below).

Figure 21. Adjusted Survival (%): Females



In the males the most common non-neoplastic cause of death was attributed to urinary tract dilatation that showed a treatment-related increase in the low, mid and high dose males (see sponsor's table below). These lesions account for the early termination of the HD male group, and were described as dilated renal pelves, distended urinary bladder and/or uteral dilatation and renal tubular cysts that were observed macroscopically with renal/uretal dilatation and chronic progressive nephropathy. All other causes of death were not dose related (sponsor's table below).

Table 64. Most Frequent Causes of Death

Sex	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose Level (mg/kg/day)	0	0	100	300	1000	0	0	100	300	1000
Number Examined	50	50	50	50	50	50	50	50	50	50
Scheduled Sacrifice	18	22	24	19	16	25	15	15	22	17
Lymphosarcoma	3	5	1	2	0	5	9	6	8	3
Cutaneous Inflammation	5	4	6	3	6	2	2	2	1	3
Gavage-Related Death	2	2	1	5	0	3	3	8	1	7
Urinary Tract Dilatation	0	0	**3**	**8**	**17**	0	0	0	0	0
Undetermined	3	1	1	1	3	2	2	2	1	4
Bronchiolar-Alveolar Carcinoma	3	4	4	2	0	0	2	0	0	0
Hemangiosarcoma	2	0	2	2	2	1	0	2	1	3
Chronic Progressive Nephropathy	0	0	0	0	0	0	3	4	5	2
Histiocytic Sarcoma	1	0	0	0	0	0	5	4	1	3
Amyloidosis	3	0	1	2	1	3	1	1	0	1

Bolded text and numbers considered BI 10773-related.

Clinical Signs

Clinical signs were generally unremarkable with the exception of swollen midline ventral abdomen that was dose-dependently increased in the empagliflozin-treated males (see sponsor's table below). In addition the incidence of non-formed feces was increased in the 1000 mg/kg males and females. The swollen midline ventral abdomen correlated with enlarged kidneys and urinary bladder distension observed macroscopically and kidney dilation, urinary bladder dilatation and occasional ureter dilatation observed microscopically.

Table 65. Clinical Signs – Males

Category / Sign	Sex:	Males				
	Group:	1	2	3	4	5
	Dose Level:	0	0	100	300	1000
	Dose Units:	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
	Number in Group:	50	50	50	50	50
		N	N	N	N	N
Swollen, Midline Ventral Abdomen		11	17	22	25	29
Feces, Nonformed		1	0	0	3	27

Table 66. Clinical Signs – Females

Category / Sign	Sex:	Females				
	Group:	1	2	3	4	5
	Dose Level:	0	0	100	300	1000
	Dose Units:	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
	Number in Group:	50	50	50	50	50
		N	N	N	N	N
Swollen, Midline Ventral Abdomen		4	15	8	12	11
Feces, Nonformed		0	0	2	3	16

Body Weights

Treatment with empagliflozin had no effect on the mean body weight in both males and females at the end of treatment (see table below). Mean body weights were, however, statistically significantly (ss) reduced in all empagliflozin-treated males for weeks 12 to 78, and did not correlate with the observed increased food consumption (vehicle 1 and 2 were combined for the analysis). The reduced mean body weight ranged from 5-10% and was not dose-dependent. Mean body weights were also ss reduced 3-7% in the 100 and 300 mg/kg males from weeks 86-94, respectively. Similar final body weight at the end of the study appears due to a downward trend in body weight of both control groups, rather than a change in the dosed groups.

In contrast, there was a statistically significant increase in body weight at weeks 2-26 in the 1000 mg/kg females (data not shown but see sponsor's figure below). The increase in body weight ranged from 5-12% and was associated with increased food consumption. However, overall body weight for the duration of the study was not statistically significantly increased. Mean body weight was also ss increased at weeks 4-13 and week 18 in the 300 mg/kg females. The increase in body weight was of a lower magnitude compared to the high dose females and ranged from 3-7%.

Figure 22. Mean Body Weight – Males

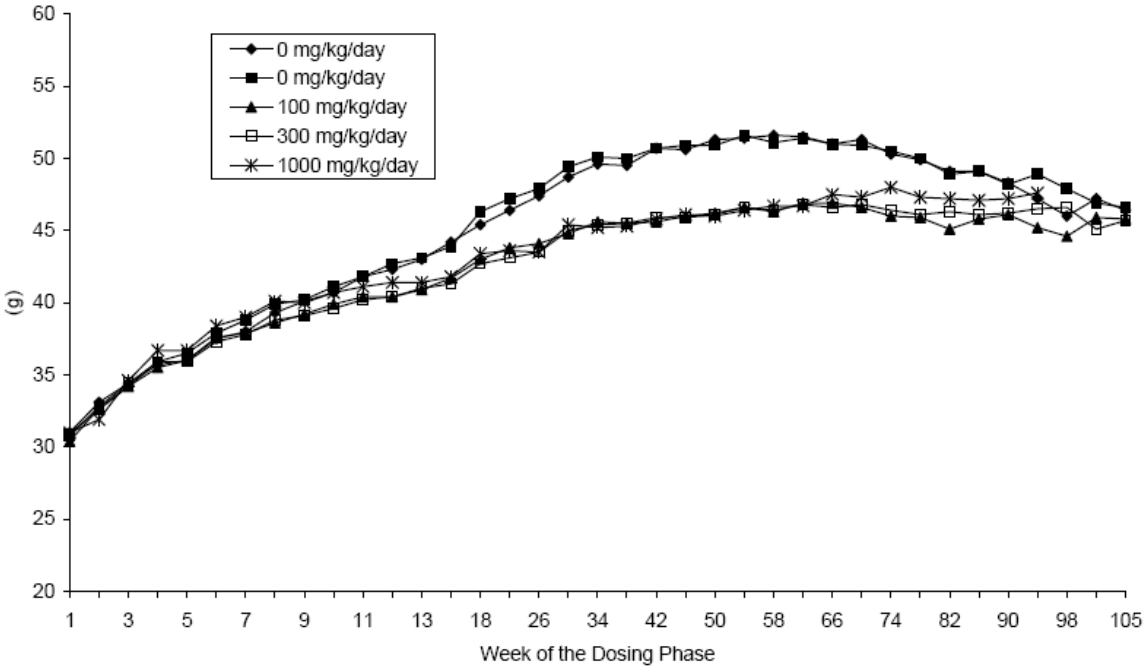
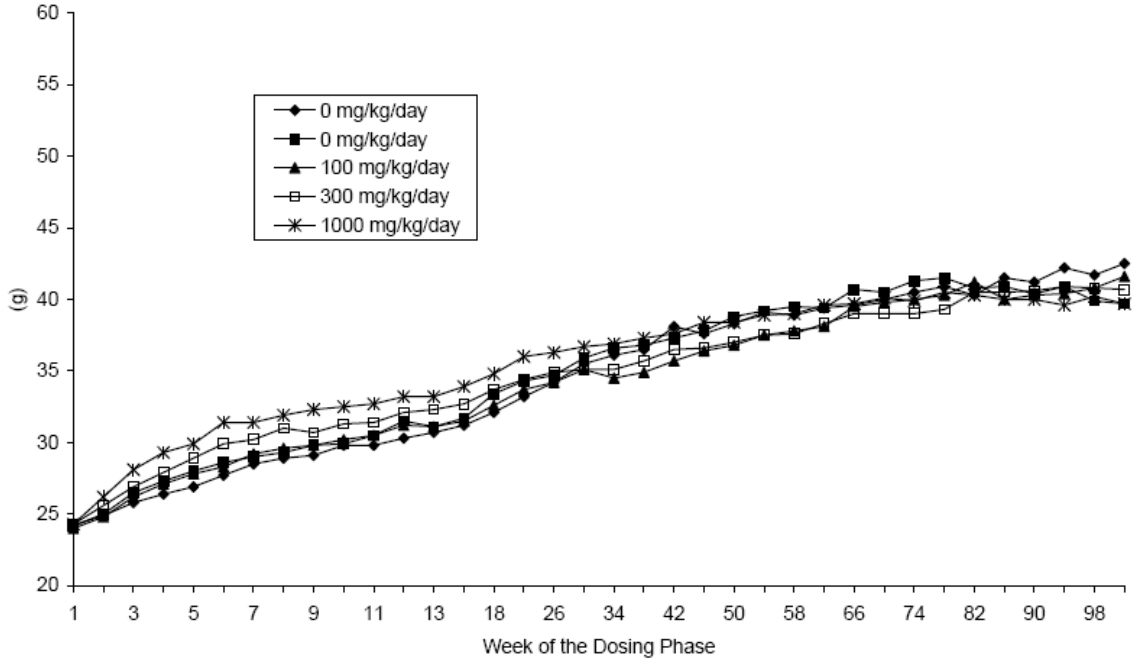


Figure 23. Mean Body Weight – Females



Body Weight Summary					
Male			Female		
Treatment mg/kg/day	BW (g)	BW Gain (g)	Treatment mg/kg/day	BW (g)	BW Gain (g)
Vehicle	46.4	14.4	Vehicle	42.5	18.6
Vehicle	46.6	16.2	Vehicle	39.7	16
100	45.8	16.2	100	41.6	16.8
300	45.7	16	300	40.7	16.2
1000	47.6#	-	1000	39.7	15.8

BW – Body weight, BW gain relative to baseline, #Week 94

Feed Consumption

In general, mean food consumption was statistically significantly increased in all empagliflozin-treated animals, particularly the mid and high dose males and the empagliflozin-treated females. The incidence and magnitude of food consumption was dose-related up to week 89 for the males and week 101 for the females, respectively. The increases ranged from 7 to 15%, 14 to 25% and 17 to 33% in the 100, 300 and 1000 mg/kg/day treated males, respectively and 6 to 23%, 12 to 27% and 17 to 45% in the 100, 300 and 1000 mg/kg/day treated females, respectively (see sponsor's figure below). The magnitude and dose dependence of increased in food consumption did not correlate with the reduced mean body weight in males, but was correlative with increased body weight in the females. **Reviewer note**: the sponsor calculated the statistical significance for food consumption using the mean of vehicle 1 and 2.

Figure 24. Mean Food Consumption – Males

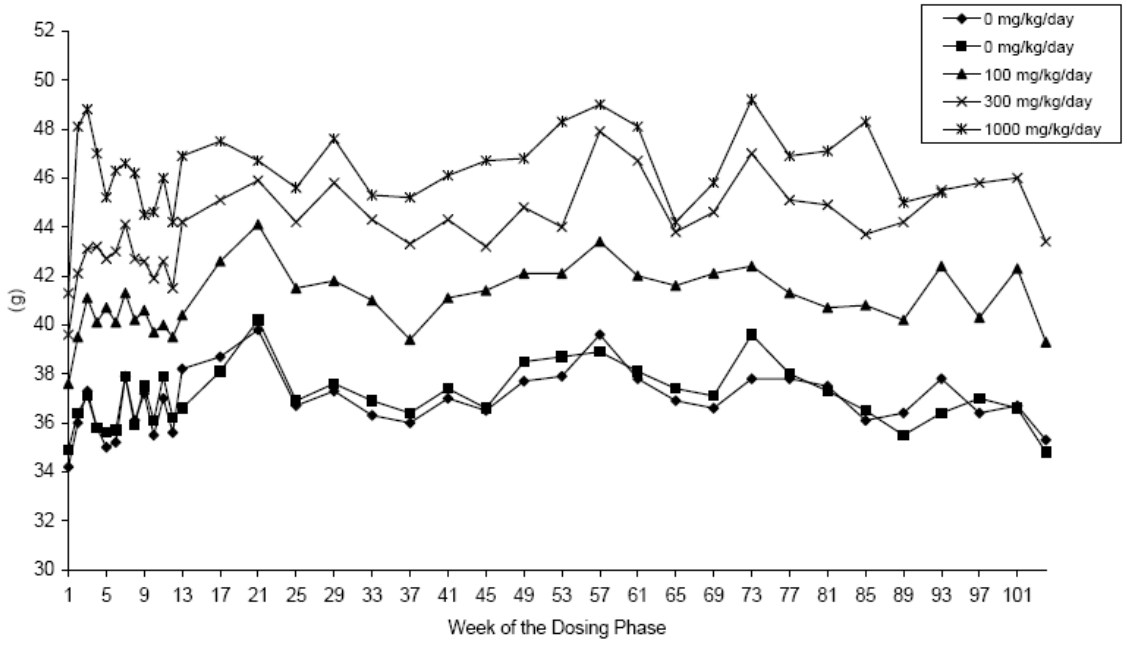
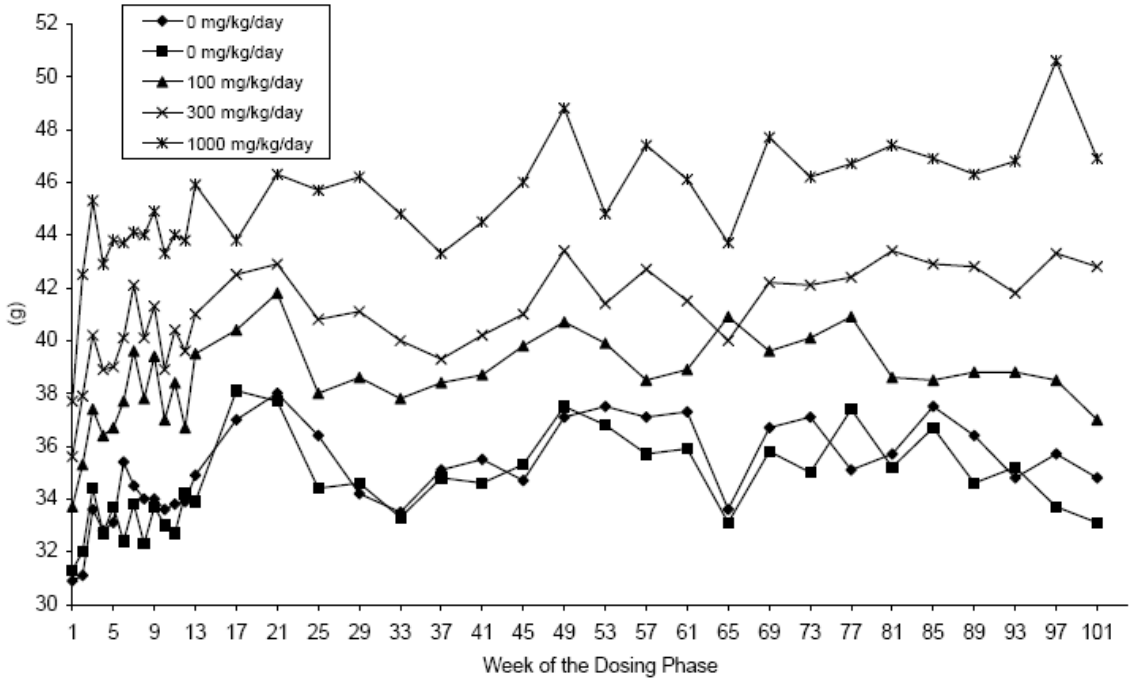


Figure 25. Mean Food Consumption – Females



Gross Pathology

Scheduled Sacrifice

An increased incidence of kidney cyst and rough surface was observed in the 1000 mg/kg males and these macroscopic observations correlated microscopically with chronic progressive nephropathy (CPN). There was also an increased incidence of large kidney in the 100 and 300 mg/kg males which correlated microscopically with kidney dilatation. The incidence of calculus and distention of the urinary bladder was also increased in the 100 and 300 mg/kg males. Distension of the urinary bladder was sometimes correlated microscopically with urinary bladder dilatation. **Reviewer note:** The incidence of large kidney and urinary bladder distension in the 1000 mg/kg males was also of higher incidence in the unscheduled sacrifice animals (see below).

Table 67. BI 10773 Gross Pathology – Scheduled Sacrifice

	-- Males --					-- Females --				
Group:	1	2	3	4	5	1	2	3	4	5
Number in group:	18	22	24	19	16	25	15	15	22	17
Kidney										
Abnormal Shape	0	0	1	0	0	0	0	0	0	0
Cyst	5	3	8	7	13	0	0	2	1	1
Discolored	0	0	3	1	1	1	0	0	0	0
Large	1	2	6	7	3	0	0	0	0	0
Rough Surface	0	0	0	0	3	0	0	0	0	0
Total:	6	5	18	15	20	1	0	2	1	1
Urinary Bladder										
Calculus	1	0	0	0	0	0	0	0	0	0
Distended	7	5	13	12	7	0	0	0	0	0
Total:	8	5	13	12	7	0	0	0	0	0

Unscheduled Sacrifice

The incidence of large kidneys was increased in the empagliflozin-treated males, with discoloration increased in the 1000 mg/kg males. Large kidneys correlated microscopically with kidney dilatation. Distension of the urinary bladder was increased in the empagliflozin-treated males and in most cases correlated microscopically with urinary bladder dilatation. Larger ureters were also indentified macroscopically in the empagliflozin-treated males and correlated microscopically with dilatation.

In addition, the incidence of urinary bladder calculus and small seminal vesicles was increased in the 300 mg/kg males and correlated microscopically with urinary bladder calculus (in some instances) and reduced secretion, respectively.

Table 68. Empagliflozin Gross Pathology – Unscheduled Sacrifice

	-- Males --					-- Females --				
Group:	1	2	3	4	5	1	2	3	4	5
Number in group:	18	22	24	19	16	25	15	15	22	17
Kidney										
Abnormal Shape	0	0	0	0	0	1	0	0	0	0
Contains Fluid	0	0	2	1	1	0	0	0	1	0
Cyst	3	1	4	6	13	1	0	0	1	1
Discolored	3	0	1	3	5	3	1	1	4	0
Large	1	3	12	11	13	1	0	0	1	0
Mass	0	0	0	0	1	0	0	0	0	0
Small	1	0	1	0	0	0	0	0	0	0
Rough Surface	0	1	1	1	2	1	1	0	2	0
Total:	8	5	21	22	35	7	2	1	9	1
Urinary Bladder										
Abnormal Contents	2	1	0	0	0	0	0	0	0	0
Calculus	1	0	0	4	0	0	0	0	0	0
Contains Fluid	1	0	2	0	0	0	0	0	0	0
Discolored	0	0	1	1	1	0	0	1	0	0
Distended	8	7	13	20	19	0	0	0	1	1
Thickened	0	0	0	1	0	0	0	0	0	0
Total:	12	8	16	26	20	0	0	1	1	1
Ureter										
Abnormal Contents	0	0	0	0	0	1	0	0	0	0
Distended	0	0	2	0	0	0	1	0	0	0
Large	0	1	3	8	6	2	0	2	1	2
Not Identified	0	0	0	1	0	1	2	2	2	1
Total:	0	1	5	9	6	4	3	4	3	3
Seminal Vesicle										
Discolored	3	2	1	4	1	0	0	0	0	0
Large	9	14	6	5	5	0	0	0	0	0
Not Identified	0	0	1	0	0	0	0	0	0	0
Small	0	0	0	4	0	0	0	0	0	0
Total:	12	16	8	13	6	0	0	0	0	0

Total Unscheduled and Scheduled Sacrifices

The incidence of large kidneys and cysts was increased in the empagliflozin-treated males, with discoloration and rough surface increased in the 1000 mg/kg males (see sponsor's table and table 12 below). Distension of the urinary bladder and large ureters was also increased in the empagliflozin-treated males. Kidney cysts and kidney rough surface correlated microscopically with chronic progressive nephropathy (CPN). Large kidneys and ureters correlated microscopically with dilatation. Distension of the urinary bladder was on occasion correlated with microscopic urinary bladder dilatation.

Table 69. Macroscopic Urinary Tract Findings

Sex	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose Level (mg/kg/day)	0	0	100	300	1000	0	0	100	300	1000
Number Examined	50	50	50	50	50	50	50	50	50	50
Kidney, Cyst	8	4	**12**	**13**	**26**	1	0	2	2	2
Kidney, Large Pelvis	2	5	**18**	**18**	**16**	1	0	0	1	0
Kidney, Rough Surface	0	1	1	1	**5**	1	1	0	2	0
Urinary Bladder, Distended	15	12	**26**	**32**	**26**	0	0	0	1	1
Ureter, Large	1	2	**5**	**10**	**6**	2	0	2	2	2

Bolded numbers considered BI 10773-related.

The incidence of discolored liver was increased in the 1000 mg/kg males but was without a defined microscopic correlate. In addition, the incidence of small seminal vesicles was increased in the 300 mg/kg males and correlated microscopically with reduced secretion (see sponsor's table below)

Table 70. BI 10773 Gross Pathology – All Unscheduled and Scheduled Sacrifices

	-- Males --					-- Females --				
Group:	1	2	3	4	5	1	2	3	4	5
Number in group:	50	50	50	50	50	50	50	50	50	50
Liver										
Abnormal Shape	0	0	0	0	1	0	0	0	0	1
Adhesion	0	0	1	0	0	0	0	0	0	0
Cyst	1	4	4	0	2	2	3	0	2	0
Depressed Area	0	0	0	0	0	1	0	0	0	1
Discolored	3	3	4	3	6	3	7	3	2	4
Large	0	0	0	1	0	1	3	2	3	4
Mass	17	12	17	13	4	1	3	2	0	1
Mottled	0	0	0	0	0	0	0	0	0	1
Not Identified	0	0	0	0	0	0	0	0	0	1
Raised Area	2	2	1	1	3	0	0	0	1	1
Rough Surface	1	0	2	0	0	0	0	1	3	1
Small	1	0	0	0	0	0	0	0	0	0
Thickened	0	0	2	0	0	0	2	0	0	0
Total:	25	21	31	18	16	8	18	8	11	15
Kidney										
Abnormal Shape	0	0	1	0	0	1	0	0	0	0
Contains Fluid	0	0	2	1	1	0	0	0	1	0
Cyst	8	4	12	13	26	1	0	2	2	2
Discolored	3	0	4	4	6	4	1	1	4	0
Large	2	5	18	18	16	1	0	0	1	0
Mass	0	0	0	0	1	0	0	0	0	0
Small	1	0	1	0	0	0	0	0	0	0
Rough Surface	0	1	1	1	5	1	1	0	2	0
Total:	14	10	39	37	55	8	2	3	10	2
Urinary Bladder										
Abnormal Contents	2	1	0	0	0	0	0	0	0	0
Calculus	2	0	0	4	0	0	0	0	0	0
Contains Fluid	1	0	2	0	0	0	0	0	0	0
Discolored	0	0	1	1	1	0	0	1	0	0
Distended	15	12	26	32	26	0	0	0	1	1
Thickened	0	0	0	1	0	0	0	0	0	0
Total:	20	13	29	38	27	0	0	1	1	1
Ureter										
Large	1	2	5	10	6	2	0	2	2	2
Not Identified	0	0	0	1	0	1	2	2	2	1
Total:	1	2	7	11	6	4	3	4	4	3
Seminal Vesicle										
Discolored	8	7	5	6	2	0	0	0	0	0
Large	18	26	11	10	10	0	0	0	0	0
Not Identified	0	0	1	0	0	0	0	0	0	0
Small	0	0	1	4	0	0	0	0	0	0
Total:	26	33	18	20	12	0	0	0	0	0

Histopathology

Peer Review: Yes

Neoplastic
Renal tubular adenoma was increased in the 300 and 1000 mg/kg males compared to vehicle controls. Renal tubular carcinoma was also increased in the 1000 mg/kg males (see sponsor's table 71 below). Relative to the combined control groups, the increase in renal tubular adenoma was statistically significant by trend ($p = 0.002$) and pair-wise ($p = 0.028$) comparison (see table 72 below). The increase in renal tubule carcinoma was not statistically significant by trend or by pair-wise testing. Combined, the increase in renal tubule adenoma and carcinoma was statistically significant by trend ($p < 0.0001$) and pair-wise testing ($p = 0.002$) (see table 72 below).

Table 71. Renal Tubule Neoplastic Findings – Male Mice

Group	1	2	3	4	5	Historical Control 1[a]	Historical Control 2[b]
Dose Level (mg/kg/day)	0	0	100	300	1000	Mean (range) [%]	Mean (range) [%]
Number Examined	50	50	50	50	50	880	540
Adenoma, Tubular							
Total Incidence	0	0	0	1	**3**	5	0
%	0.0	0.0	0.0	2.0	**6.0**	0.6 (0.0-2.9)	0.0 (0.0-0.0)
Carcinoma, Tubular							
Total Incidence	0	0	0	0	**2**	1	0
%	0.0	0.0	0.0	0.0	**4.0**	0.1 (0.0-1.7)	0.0 (0.0-0.0)
Adenoma & Carcinoma							
Total Incidence	0	0	0	1	**5**	6	0
%	0.0	0.0	0.0	2.0	**10**	0.7 (0.0-2.9)	0.0 (0.0-0.0)
Hyperplasia, Tubular, Cystic							
Minimal	19	17	**22**	**22**	**14**		
Slight	1	2	**5**	**12**	**15**		
Moderate	0	0	0	**2**	**8**		
Total Incidence	20	19	**27**	**36**	**37**	NA	NA
%	40	38	**54**	**72**	**74**		
Hyperplasia, Tubular, Atypical							
Minimal	0	0	0	0	**8**		
Slight	0	0	0	0	**3**		
Total Incidence	0	0	0	0	**11**	NA	NA
%	0.0	0.0	0.0	0.0	**22**		
Hyperplasia, Tubular Epithelium/Tubule Cell							
Total Incidence	NA	NA	NA	NA	NA	3	2
%						0.3 (0.0-3.3)	0.4 (0.0-1.7)

Bolded numbers considered BI 10773-related.
NA = Not applicable
a (b) (4) data from twenty-four 104-week CD-1 mice carcinogenicity studies with end dates from November 1993 to August 2006.
b (b) (4) data from eight 104-week CD-1 mice carcinogenicity studies with end dates from March 2009 to June 2011.

The incidence and severity (minimal to moderate) of renal cystic tubular hyperplasia was dose-dependently increased in the empagliflozin-treated males. The incidence of a pre-neoplastic lesion, renal atypical tubular hyperplasia (minimal to slight) was also increased in the 1000 mg/kg males (see sponsor's table 71 above). Renal cystic tubular hyperplasia (minimal to slight) was also slightly increased in the empagliflozin-treated females (see sponsor's table 73 below). Atypical tubular hyperplasia (slight) was present in one 300 mg/kg female (see sponsor's table 73 below).

Table 72. Summary of Mouse Tumor Statistics

Summary of tumors with any significant difference from controls †									
Tissue/Tumor	Sex	Empagliflozin (mg/kg/day)					Statistics (p-value) [a]		
		0 (vehicle 1)	0 (vehicle 2)	100	300	1000	Trend	Pair-wise Mid-dose	Pair-wise High-dose
Kidney: adenoma, tubular	M	0	0	0	1	3	0.002	nss	0.028
Kidney: malignant carcinoma, tubular	M	0	0	0	0	2	nss	NA	nss
Kidney: adenoma & carcinoma combined	M	0	0	0	1	5	<0.0001	nss	0.002

† Statistical analyses summarized from FDA statistics review (Dr. Min)
F = female, M = male, nss = not statistically significant, NA = not applicable
[a] Statistical analysis with vehicle 1 and vehicle 2 combined

Table 73. Renal Tubule Neoplastic Findings – Female Mice

Group	1	2	3	4	5	Historical Control 1[a]	Historical Control 2[b]
Dose Level (mg/kg/day)	0	0	100	300	1000	Mean (range) [%]	Mean (range) [%]
Number Examined	50	50	50	50	50	1047	540
Adenoma, Tubular							
Total Incidence	0	0	0	0	0	0	1
%	0.0	0.0	0.0	0.0	0.0	0.0 (0.0-0.0)	0.2 (0.0-1.7)
Carcinoma, Tubular							
Total Incidence	0	0	0	0	0	0	0
%	0.0	0.0	0.0	0.0	0.0	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Hyperplasia, Tubular, Cystic							
Minimal	3	4	6	5	8		
Slight	0	0	2	1	0		
Total Incidence	3	4	8	6	8	NA	NA
%	6.0	8.0	16	12	16		
Hyperplasia, Tubular, Atypical							
Slight	0	0	0	1	0		
Total Incidence	0	0	0	1	0	NA	NA
%	0.0	0.0	0.0	2.0	0.0		
Hyperplasia, Tubular Epithelium/Tubule Cell							
Total Incidence	NA	NA	NA	NA	NA	3	2
%						0.3 (0.0-3.3)	0.4 (0.0-1.7)

NA = Not applicable

a (b) (4) data from twenty-four 104-week CD-1 mice carcinogenicity studies with end dates from November 1993 to August 2006.

b (b) (4) data from eight 104-week CD-1 mice carcinogenicity studies with end dates from March 2009 to June 2011.

In females, increased incidence of significant neoplastic findings were found for body, whole/cavity histiocytic sarcoma (p =0.0037), bronchiolar-alveolar lung carcinoma (p = 0.0182) and body, whole cavity lymphosarcoma (p = 0.0194) in vehicle control 2 compared to vehicle control 1 (see sponsor's table below). In addition, body, whole/cavity histiocytic sarcoma was increased and statistically significant in the 100 mg/kg (p = 0.0064) and the 1000 mg/kg females (p = 0.0116) when compared to vehicle control 1. Similarly, body, whole/cavity lymphosarcoma was increased and statistically significant in the 300 mg/kg females (p = 0.0361) when compared to vehicle control 1 (see sponsor's table below). These neoplastic findings are considered to be incidental.

Table 74. Sponsor's Statistical Analysis of Neoplastic Lesions – Female Mice

		Unadjusted Lifetime Incidence Rate				
	Group	1	2	3	4	5
Tissue and Lesion	Dose level (mg/kg/day)	0	0	100	300	1000
Lung, M-Carcinoma, Bronchiolar-Alveolar (I/F)		1/50	6/50	2/50	0/50	1/50
Group 1 vs. Group 2 (one-sided)		NA	0.0182+*	NA	NA	NA
Lung, B-Adenoma, Bronchiolar-Alveolar (I) / M-Carcinoma, Bronchiolar-Alveolar (I/F)		5/50	7/50[a]	7/50	6/50	6/50
Group 1 vs. Group 2 (one-sided)		NA	0.2083+	NA	NA	NA
Gland, Harderian, B-Ademona (I/F)		4/50	2/50	3/50	0/50	1/50
Group 1 vs. Group 2 (one-sided)		NA	0.3366-	NA	NA	NA
Mammary, Female, M-Carcinoma (I/F)		0/46	2/45	0/42	0/42	0/47
Group 1 vs. Group 2 (one-sided)		NA	0.2051+(E)	NA	NA	NA
Uterus, B-Polyp, Endometrial Stromal (I)		4/50	2/50	2/50	2/50	1/50
Group 1 vs. Group 2 (one-sided)		NA	0.2313-	NA	NA	NA
Uterus, M-Carcinoma, Endometrial (I)		Combined incidence = 0/100		0/50	0/50	2/50
Groups 1 and 2 combined vs. Groups 3-5 (one-sided)		0.0461+#(E)[b]		NA	NA	0.1175+(E)
Body, Whole/Cavity, M-Hemangiosarcoma (I/F)		3/50	1/50	3/50	1/50	4/50
Group 1 vs. Group 2 (one-sided)		NA	0.4149-(E)	NA	NA	NA
Groups 1 and 2 combined vs. Groups 3-5 (one-sided)		Combined incidence = 4/100; p-value=0.1006+		NA	NA	0.0763+
Body, Whole/Cavity, M-Histiocytic Sarcoma (I/F)		1/50	7/50	6/50	3/50	6/50
Group 1 vs. Group 2 (one-sided)		NA	0.0037+**	NA	NA	NA
Group 1 vs. Groups 3-5 (one-sided)		0.0548+[b]	NA	0.0064+**	0.2464+(E)	0.0116+#
Body, Whole/Cavity, M-Lymphosarcoma (I/F)		9/50	14/50	12/50	16/50	6/50
Group 1 vs. Group 2 (one-sided)		NA	0.0194+*	NA	NA	NA
Group 1 vs. Groups 3-5 (one-sided)		0.2467-[b]	NA	0.0551+	0.0361+*	NA
Group 2 vs. Groups 3-5 (one-sided)		NA	0.0262-#[b]	NA	0.4037-	NA

I = Incidental tumor; F = Fatal tumor; (E) = Exact test; NA = Not analyzed.
+ = Effect in the increased direction; - = Effect in the decreased direction.
* = Significant at 0.05 level; ** = Significant at 0.01 level.
# = Not a significant trend at the 0.005 level or a significant increase at the 0.01 level for common tumors.
a The following animals had both B-Adenoma and M-Carcinoma: Animal Nos. A26642, A26657, A26687, and A26688 (Group 2 females). For the purpose of the statistical analysis, each animal was counted once.
b p-value for trend analysis.

Non Neoplastic

Kidney

A pathology working group (PWG) identified an increased incidence of minimal single cell necrosis of the proximal convoluted tubules in the 1000 mg/kg males (see sponsor's table below). Single cell necrosis is suggestive of chronic low level injury occurring in the kidney.

Minimal to moderate karyomegaly of segment 2 proximal convoluted tubules was present in the 1000 mg/kg males. Minimal karyomegaly of segment 3 proximal convoluted tubules was also identified in the empagliflozin-treated males (see sponsor's table below). Tubular epithelial cells with karyomegaly were generally also hypertrophic and this correlates with the tubular hypertrophy observed. Karyomegaly is considered a response to a toxic insult (Hard et.al., 1999).

Minimal to slight, focal or multifocal tubular cell hypertrophy was present in the empagliflozin-treated females and in particular, in the 300 and 1000-mg/kg males (see sponsor's tables below). Chronic progressive nephropathy (CPN) was present in all mice. However, the CPN severity (moderate to marked) was increased in the 1000-mg/kg males and treatment with empagliflozin appears to exacerbate the underlying disease in the males.

Minimal to slight cystic dilatation of cortical tubules or the Bowman's capsule was present in the 100 and 300 mg/kg males. Increased incidence and severity (minimal to marked) of cystic tubular dilatation was noted in the 1000 mg/kg males (see sponsor's table below). Cystic dilatation of cortical tubules occurred in concordance with renal pelvic dilatation, urinary bladder dilatation and ureter dilatation (see ureter and urinary bladder section below).

Atrophy of the proximal convoluted tubules with slight to moderate severity was observed in the 100 and 300-mg/kg males. Minimal to marked severity of atrophy of the proximal convoluted tubules was present in 1000 mg/kg males (see sponsor's table below). The incidence and severity of renal pelvic dilation (minimal to marked) also increased in the empagliflozin-treated males (see sponsor's table below).

Hard et.al., 1999: Non-proliferative Lesions of the Kidney and Lower Urinary Tract in Rats: Guides For Toxicologic Pathology, STP/ARP/AFIP, Washington DC

Table 75. Microscopic Findings in the Kidney

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose Level (mg/kg/day)	0	0	100	300	1000	0	0	100	300	1000
Number Examined	50	50	50	50	50	50	50	50	50	50
Single Cell Necrosis, Tubular										
Minimal	9	8	9	8	**29**	1	0	0	0	0
Total Incidence	9	8	9	8	**29**	1	0	0	0	0
Karyomegaly, Tubular, Segment 2										
Minimal	0	2	0	2	**22**	0	0	0	0	0
Slight	0	0	0	0	**14**	0	0	0	0	0
Moderate	0	0	0	0	**2**	0	0	0	0	0
Total Incidence	0	2	0	2	**38**	0	0	0	0	0
Karyomegaly, Tubular, Segment 3										
Minimal	0	0	**5**	**3**	**9**	0	0	0	0	0
Total Incidence	0	0	**5**	**3**	**9**	0	0	0	0	0
Hypertrophy, Tubular										
Minimal	8	5	5	**18**	**13**	2	2	**9**	7	7
Slight	0	0	0	0	0	0	1	1	0	1
Total Incidence	8	5	5	**18**	**13**	2	3	10	7	8
Chronic Progressive Nephropathy										
Minimal	20	28	24	15	**12**	25	24	27	27	25
Slight	13	10	19	25	**11**	3	5	**6**	4	2
Moderate	7	3	3	3	22	3	9	**6**	5	3
Marked	0	1	2	0	1	1	0	0	1	0
Total Incidence	40	42	48	43	46	32	38	39	37	30
Cysts[a]										
Minimal	0	0	4	4	**6**	0	0	0	1	0
Slight	0	0	2	2	**3**	0	0	1	0	0
Moderate	0	0	0	0	**4**	0	0	0	0	0
Marked	0	0	0	0	**4**	0	0	0	0	0
Total Incidence	0	0	**6**	**6**	**17**	0	0	1	1	0
Atrophy, Tubular										
Minimal	0	0	0	0	**6**	0	0	0	0	0
Slight	0	0	2	1	**4**	0	0	0	0	0
Moderate	0	0	0	1	**5**	0	0	0	0	1
Marked	0	0	0	0	**5**	0	0	0	0	0
Total Incidence	0	0	2	2	**20**	0	0	0	0	1
Dilatation, Pelvis										
Minimal	3	4	**8**	**4**	**6**	0	1	0	4	2
Slight	2	3	**12**	**10**	**12**	1	0	0	0	0
Moderate	0	0	**6**	**12**	**10**	0	0	0	1	1
Marked	0	0	0	0	0	0	0	0	1	0
Total Incidence	5	7	**26**	**26**	**28**	1	1	0	**6**	3

Bolded numbers considered BI 10773-related.

a Cysts were diagnosed only when greater in overall number/severity than the maximal control.

Ureter and Urinary Bladder

Ureter dilatation with a dose-dependent increase in severity (minimal to marked) and severity was observed in the empagliflozin-treated males (see sponsor's table below). Urinary bladder transitional cell hyperplasia and inflammation was also increased in the 300 mg/kg males (see sponsor's table below). **Reviewer note:** ureter dilatation and urinary bladder dilatation are likely related to secondary pharmacology due to polyuria which is a known pharmacodynamic effect for the SGLT2 inhibitor class.

Table 76. Microscopic Findings in the Ureter and Urinary Bladder

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose Level (mg/kg/day)	0	0	100	300	1000	0	0	100	300	1000
Ureter										
Number Examined	48	48	49	49	48	50	48	49	49	47
Dilatation										
Minimal	4	1	**2**	**2**	**6**	0	0	0	1	0
Slight	1	1	**5**	**5**	**7**	0	0	0	0	0
Moderate	0	3	**8**	**13**	**8**	0	0	0	2	0
Marked	0	0	**2**	**4**	**5**	0	0	0	0	0
Total Incidence	5	5	**17**	**24**	**26**	0	0	0	3	0
Urinary Bladder										
Number Examined	50	50	49	50	50	50	50	50	50	50
Dilatation										
Present	15	12	**22**	**27**	**23**	0	0	0	1	1

Bolded numbers considered BI 10773-related.

Table 77. Summary of Microscopic Observations – Bladder

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --					-- Animals Affected -- Females --				
Tissues With Diagnoses	Dosage group: / No. in group:	Ctls 50	2 50	3 50	4 50	5 50	Ctls 50	2 50	3 50	4 50	5 50
Urinary Bladder	Number examined:	50	50	49	50	50	50	50	50	50	50
	Unremarkable:	32	33	24	20	25	48	46	42	39	46
Dilatation		15	12	22	27	23	0	0	0	1	1
Hyperplasia, Transitional Cell		3	3	2	7	2	0	0	0	1	0
C-Hematopoietic Neoplasm, see Body, Whole for type		1	4	0	1	1	2	4	7	9	3
Calculus		0	0	0	2	0	0	0	0	0	0
Hemorrhage		1	0	0	0	0	0	0	0	0	0
Inflammation		3	4	5	10	4	0	0	0	1	0
Inflammation, Vessel		0	0	0	0	0	0	0	1	0	0

Liver

Increased cystoplasmic vacuolation (minimal to moderate) was observed in the empagliflozin-treated males and females and severity was dose-dependently increased in the females (see sponsor's table below).

Table 78. Microscopic Findings in the Liver

	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Dose Level (mg/kg/day)	0	0	100	300	1000	0	0	100	300	1000
Number Examined	50	50	50	50	50	50	50	50	50	50
Liver										
Increased Cytoplasmic Vacuolation										
Minimal	0	2	8	13	13	2	0	4	7	7
Slight	0	0	0	2	2	1	0	1	9	6
Moderate	0	0	0	0	0	0	0	0	0	4
Total Incidence	0	2	**8**	**15**	**15**	3	0	5	**16**	**17**

Bolded numbers considered BI 10773-related.

Toxicokinetics

In this 2-year mouse carcinogenicity study the sponsor administered empagliflozin at 100, 300 and 1000 mg/kg in males and females, respectively. Blood was obtained from satellite TK animals at days 1 (pre-dose), 88 and 179. C_{max} and AUC_{0-24} showed no consistent trend (dose-proportionality) but appeared to be saturated for C_{max} at 1000 mg/kg. Systemic exposure in the males was lower (1.4-2.6-fold) compared to the female animals (sponsor's table below).

Table 79. Mouse Carcinogenicity Toxicokinetics

Toxicokinetic Parameters of BI 10773 after Oral Administration of BI 10773 XX during a 104-Week Oral Toxicity Study in CD-1 Mice					
TK Parameter	Sex	Drug Day	BI 10773 XX (mg/kg/day)		
			100	300	1000
C_{max} (nM)	Male	Day 1	8,210	24,900	34,500
		Day 88	6,800	24,100	30,600
		Day 179	6,730	12,200	21,300
	Female	Day 1	16,200	42,300	58,300
		Day 88	13,100	32,300	65,400
		Day 179	9,030	35,000	68,200
AUC_{0-24} (nM·h)	Male	Day 1	24,800	71,800	105,000
		Day 88	17,800	55,200	231,000
		Day 179	20,700	51,700	211,000
	Female	Day 1	42,700	103,000	194,000
		Day 88	28,100	82,300	377,000
		Day 179	32,700	135,000	296,000
t_{max} (h)	Male	Day 1	1	1	1
		Day 88	1	1	1
		Day 179	1	1	2
	Female	Day 1	1	1	1
		Day 88	1	1	2
		Day 179	1	2	2

Dosing Solution Analysis

The formulations were stable for 24 hr when stored at room temperature, and for 18 days when refrigerated at 2-8 °C (results not shown). The concentration verification throughout the study were within 10% of the nominal concentration and were considered homogeneous (results not shown).

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

A GLP-compliant fertility study was conducted in the rat. The study report was fully reviewed and is summarized here.

Study of Fertility and Early Embryonic Development to Implantation of BI 10773 XX in Rats

Study no.:	08R008, U09-3133-01
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 8th 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 10773 XX, BI10773XXN07 and 100%

Methods

Doses:	0, 100, 300 and 700 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	PO (gavage)
Formulation/Vehicle:	0.5% hydroxyethylcellulose in water
Species/Strain:	Wistar (Han)
Number/Sex/Group:	25/sex/group
Satellite groups:	No
Study design:	Males treated 4 weeks prior to mating through determination of female fertility (approx. 7 weeks total); Females treated 2 weeks prior to pairing through to GD 7 with laparohysterectomy at GD 15.
Deviation from study protocol:	None that affected study outcome

Key Study Findings

- Mortality was observed in one 700 mg/kg male at day 60 post-dose. The cause of death was not identified.
- Body weight gain was reduced greater than 10% in the 300 and 700 mg/kg males in the pre-mating period and also 14-27% in all treated males at the end of the study.
- Body weight gain was reduced 43% at GD 0-7 in the 700 mg/kg females.
- Blood for TK was not collected, however the doses evaluated were identical to those that were used in the rat embryofetal development study, and thus represent 22x (100 mg/kg), 48x (300 mg/kg) and 155x (700 mg/kg) MRHD,
- Due to reduced body weight gain the NOAEL for paternal toxicity is 300 mg/kg which is approximately 48x MRHD.
- Due to reduced gestational body weight gain the NOAEL for maternal systemic toxicity is 300 mg/kg and this is approximately 48x MRHD.
- There were no effects on fertility or reproductive performance in males or females and the NOAEL for fertility is 700 mg/kg; which is approximately 155x MRHD.

9.2 Embryonic Fetal Development

GLP-compliant embryo-fetal development studies (dose-range finding and definitive studies) were investigated in the rat and rabbit. All study reports were fully reviewed and are summarized here.

A Dose Range-Finding Study of the Effects of BI 10773 XX on Embryo/Fetal Development in Rats

Study no.:	07R029, U08-3562-01
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 5th 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 10773 XX, BI10773XXF07 and 99.5%

Methods

Doses:	0, 30, 100, 300 and 700 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	PO (oral gavage)
Formulation/Vehicle:	0.5% hydroxyethylcellulose in water
Species/Strain:	Rat/Wistar (Han)
Number/Sex/Group:	8/F/group
Satellite groups:	3 control F and 6/F/group
Study design:	GD 6 - GD 17
Deviation from study protocol:	None that affected the study outcome

Key Study Findings

- The NOAEL for maternal toxicity was 300 mg/kg (48x the 25 mg MRHD) due to the reduced body weight/ body weight gain and reduced food consumption at 700 mg/kg.
- The NOAEL for embryo/fetal development was 700 mg/kg (154x the 25 mg MRHD) due to lack of embryo-fetal malformations or variations.
- Wet yellow material in the anogenital area and soft stool that was noted intermittently in all HD dams during treatment.
- Body weight gain was significantly reduced in the 700 mg/kg dams at GD 6-9 and GD 6-18, respectively; and was associated with reduced food consumption in the same animals at GD 6-7, 7-8, 8-9 and 6-9, respectively.
- No effects on intrauterine growth and survival, malformations or developmental variations were found in the treated fetuses.
- Empagliflozin at 30, 100, 300 and 700 mg/kg represent 4x, 22x, 48x and 154x MRHD (25 mg), respectively.

A Study of the Effects of BI 10773 XX on Embryo/Fetal Development in Rats

Study no.:	07R030, U08-3556-01
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 4th 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 10773 XX, BI10773XXF07 and 99.5%

Methods

Doses:	0, 100, 300 and 700 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	PO (oral gavage)
Formulation/Vehicle:	0.5% hydroxyethylcellulose in water
Species/Strain:	Rat/Wistar (Han)
Number/Sex/Group:	25/F/group
Satellite groups:	None
Study design:	GD 6 - GD 17
Deviation from study protocol:	None that affected the study outcome

Key Study Findings

- The NOAEL for maternal toxicity was 100 mg/kg (22x the 25 mg MRHD) due to the reduced body weight gain and reduced food consumption at ≥ 300 mg/kg.
- The NOAEL for embryo/fetal development was 300 mg/kg (48x the 25 mg MRHD) due to the bent limb bone malformation at 700 mg/kg.
- Exposure in the dams was approximately 22x, 48x and 154x MRHD (25 mg) from the dose range-finding study with identical empagliflozin exposures.
- Yellow material on various surfaces of the body and soft stool that was noted in 11 HD females intermittently during the treatment phase.
- Mean body weight was reduced from GD 15-20 in the 300 mg/kg dams and from GD 6-20 in the 700 mg/kg dams, respectively.
- Mean body weight was also dose-dependently reduced in the 300 and 700 mg/kg dams from GD 10-20.
- Dose-dependent decreases in body weight gain was observed in the 300 and 700 mg/kg dams during the treatment period (GD 6-18) and was associated with decreased food consumption in the same animals.
- A malformation of bent limb bone was observed in 0(0), 1(1), 1(1) and 4(3) fetuses (litters) for each of the control, 100, 300 and 700 mg/kg animals, respectively.
- A vertebral/rib anomaly (wavy rib) was observed in 1(1) fetus (litter) in the 300 mg/kg group.

A Dose Range-Finding Study of the Effects of BI 10773 XX on Embryo/Fetal Development in Rabbits

Study no.:	07R031, U08-3555-01
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 5th 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 10773 XX, BI10773XXF07 and 99.5%

Methods

Doses:	0, 30, 100, 300 and 700 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	PO (oral gavage)
Formulation/Vehicle:	0.5% hydroxyethylcellulose in water
Species/Strain:	Rat/Wistar (Han)
Number/Sex/Group:	6/F/group
Satellite groups:	3/F/group empagliflozin treated for TK
Study design:	GD 7 - GD 20
Deviation from study protocol:	None that affected the study outcome

Key Study Findings

- The NOAEL for maternal toxicity was 300 mg/kg (128x the 25 mg MRHD) due to the abortion in one 700 mg/kg group dam at GD 23 that was associated with reduced food consumption and reduced body weight during the treatment phase.
- The NOAEL for embryo/fetal development was 700 mg/kg (139x the 25 mg MRHD) due to lack of embryo-fetal malformations or variations.
- Reduced defecation was noted in all 700 mg/kg dams.
- Mean body weight was reduced during GD 7-21 in the 700 mg/kg dams and was associated with reduced food consumption at GD 12-13 and 14-15, respectively.
- No effects on intrauterine growth and survival, malformations or developmental variations were found in the treated fetuses.
- Empagliflozin at 30, 100, 300 and 700 mg/kg represent 8x, 40x, 128x and 139x MRHD (25 mg), respectively.

A Study of the Effects of BI 10773 XX on Embryo/Fetal Development in Rabbits

Study no.:	07R032, U08-3564-01
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 4 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 10773 XX, BI10773XXF07 and 99.5%

Methods

Doses:	0, 100, 300 and 700 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	PO (oral gavage)
Formulation/Vehicle:	0.5% hydroxyethylcellulose in water
Species/Strain:	Rat/Wistar (Han)
Number/Sex/Group:	22/F/group
Satellite groups:	3/F/group empagliflozin treated for TK
Study design:	GD 7 - GD 20
Deviation from study protocol:	None that affected the study outcome

Key Study Findings

- The NOAEL for maternal toxicity was 300 mg/kg (128x the 25 mg MRHD) due to abortion in three 700 mg/kg group dams that was associated with reduced body weight gain during the treatment phase.
- Two of the three 700 mg/kg females that aborted had completely resorbed litters.
- The NOAEL for embryo/fetal development was 300 mg/kg (128x the 25 mg MRHD) due to the reduced number of viable fetuses (post-implantation loss due to early resorptions) at 700 mg/kg.
- Exposure in the dams was approximately 40x, 128x and 139x MRHD (25 mg) from the dose range-finding study with identical empagliflozin exposures.
- Reduced defecation was noted in all 700 mg/kg dams.
- Mean body weight gain was reduced 38, 30 and 62 g at GD 7-10 in the 100, 300 and 700 mg/kg dams, respectively.
- An approximately 3-fold body weight gain in the 700 mg/kg dams occurred in the post-treatment GD 21-24 and correlated with increased food consumption in all treatment groups at GD 21-29.
- The mean % per litter proportion of post-implantation loss due to increased early resorptions was increased in the 700 mg/kg fetuses and led to a reduced number of viable fetuses in the same group.
- No effects on malformations or developmental variations were found in the treated fetuses.

9.3 Prenatal and Postnatal Development

Two GLP compliant pre- and post-natal development studies were conducted in the rat. All study reports were fully reviewed and are summarized here.

A Study of the Effects of BI 10773 XX on Pre- and Postnatal Development Including Maternal Function in Rats

Study no.:	08R009, U09-3620-01
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 2 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 10773 XX, BI 10773XXN07 and 100%

Methods

Doses:	0, 100, 300 and 700 mg/kg
Frequency of dosing:	GD 6 to LD20
Dose volume:	10 mL/kg
Route of administration:	PO (gavage)
Formulation/Vehicle:	0.5% hydroxyethylcellulose in water
Species/Strain:	Rat/Wistar (Han)
Number/Sex/Group:	25/F/group
Satellite groups:	No
Study design:	Standard Segment III study design
Deviation from study protocol:	None.

Key Study Findings

F_0 Generation:
Maternal Toxicity:

- Weight gain during gestation (GD 6-20) was suppressed 9% to 18% at 300 and 700 mg/kg. Weight gain rebounded above control at these doses later in the lactation phase (LD 14-17) despite continued drug treatment. The 100 mg/kg dose had no clear effect on the dams, and is considered the NOAEL for maternal toxicity.

F_1 Generation

- At birth, male pups had slightly lower BW at 700mg/kg, but female pups were similar to control. BW gain of pups was suppressed at all doses during the lactation period, and survival to PND 21 was slightly less at 700 mg/kg. Physical exam noted 'small stature' of pups at the 700 mg/kg dose.

- Developmental endpoints were not conducted because the sponsor terminated the study between PND 19-24

Reviewer Comment: The sponsor terminated the study early due to significant weight loss in all of the empagliflozin-exposed pups. This is clear evidence that drug is passed to pups in the milk and has an adverse pharmacodynamic effect on the pups. Empagliflozin was not evaluated in the maternal milk. The sponsor repeated the pre- and postnatal study with empagliflozin at lower doses of 0, 10, 30 and 100 mg/kg (see below)

A Study of the Effects of BI 10773 XX on Pre- and Postnatal Development Including Maternal Function in Rats

Study no.:	08R079, U09-3711-01
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 4 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BI 10773 XX, BI 10773XXN07 and 100%

Methods

Doses:	0, 10, 30 and 100 mg/kg
Frequency of dosing:	Daily GD6 to LD20
Dose volume:	10 mL/kg
Route of administration:	PO (oral gavage)
Formulation/Vehicle:	0.5% hydroxyethyl cellulose in water
Species/Strain:	Rat/Wistar (Han)
Number/Sex/Group:	25/F/group
Satellite groups:	8F/group for milk collection and 4/F/ group for TK assessment
Study design:	8F/group used for milk collection, 4F group for TK blood at GD 17 and LD 18
Deviation from study protocol:	None that affected study outcome

Key Study Findings

F_0 Generation

- No maternal toxicity was observed. The NOAEL for maternal F_0 toxicity was 100 mg/kg which is 16x MRHD.

F_1 Generation

- Dose-dependent reduced body weight and body weight gain, particularly in high dose animals during weaning, due to lactational exposure to empagliflozin. This results in reduced mean body weight in the post-weaning period in the 100 mg/kg animals.

- At PND 21 (non-selected) pups (litters) with dilated pelvis determined macroscopically was 5(2), 0(0) and 2(2) in the 10, 30 and 100 mg/kg groups. Empagliflozin-treated F_1 females not selected for breeding also had dilated pelvi at necropsy. Empagliflozin-treated males and 2 control males not selected for breeding also had dilated pelvi at necropsy. However, unlike another SGLT2 inhibitor (dapagliflozin) this appears to be a background finding due to the low incidence.

- At PND 22 the 100 mg/kg F_1 males had a deficit of learning and memory. For learning this included an increase in time to escape and an increase in the number of errors in the Biel maze. For memory this resulted in no improvement in the time to escape the Biel maze. The learning and memory deficit was not present at PND 62.

- Empagliflozin had no effect on F_1 mating or reproductive performance and there were no morphological changes in the F_2 pups.

- The NOAEL for F_1 reproductive toxicity and F_2 toxicity was 100 mg/kg which is 16x MRHD. The NOAEL for F_1 toxicity was 30 mg/kg which is 4x MRHD.

10 Special Toxicology Studies

Placental Transfer and Lacteal Excretion of [14C]-BI 10773 Following Administration of a Single Oral Dose to Pregnant or Lactating Rats

Study no.:	DM-09-1002, U09-3767-01
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 10 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	[14C]BI 10773 XX, MH-101326-052-1 and 99.2%. BI 10773 , BI 10773XX N07 and purity not specified

Methods

Doses:	5 mg/kg
Frequency of dosing:	Once: GD 13 or GD 18; Twice: PND 11 and 12
Dose volume:	5 mL/kg
Route of administration:	PO (oral gavage)
Formulation/Vehicle:	Polyethylene glycol (PEG) 400
Species/Strain:	Rat/Wistar (Han)
Number/Sex/Group:	5/F/groups 1 & 2 and 15/F/group 3
Satellite groups:	None
Study design:	From Sponsor's submission: The tissue distribution and lacteal excretion of radioactivity were assessed following a single oral administration of [14C]-BI 10773 to timed-pregnant and lactating Wistar Hanover rats. Rats received a single oral 5-mg/kg dose of [14C]-BI 10773 at a dose volume of 5 mL/kg. Blood was collected at specified times from one animal/time point from Gestation Day 13 and 18 rats. The rats were sacrificed following blood collection and examined by quantitative whole-body autoradiography (QWBA). Milk and blood were collected from 3 lactating animals/time point dosed approximately 12 days after parturition. Blood, plasma, and milk were analyzed by liquid scintillation counting (LSC).
Deviation from study protocol:	None that affected study outcome

Key Study Findings

Group 1 Gestation Day 13

Fetal Tissues

- Radioactivity was not found in the amniotic fluid and the fetus but was found in the amniotic sac (1-24 hours post-dose).

Maternal Tissues

- Placental tissue contained empagliflozin at all time points examined.

- The peak concentration of radioactivity in the blood and plasma was 335 and 533 ng equivalents of [^{14}C]-empagliflozin/g (742 and 1180 nM), respectively at 2 hours post-dose and were quantifiable up to 24 hours post-dose.

- The blood: plasma concentration ranged from 0.619-0.683, suggesting empagliflozin and its metabolites were more associated with plasma rather than the blood and its components.

- The distribution of [^{14}C]-empagliflozin was limited with radioactivity quantifiable in each tissue, but not all time points, with the exception of the bone, cerebellum, cerebrum, eye, eye lens, abdominal fat, medulla, olfactory lobe and spinal cord where no radioactivity was found.

- The highest concentration of radioactivity was found in the stomach contents and the small intestine contents consistent with oral dose administration.

- The tissue: plasma concentration ratio was less than one for most tissues. However, the tissue: plasma concentration ratio were also high in the bile (≥20), cecum (≥3 at 2h post-dose), kidney (including cortex and medulla) (≥3), liver (≥8) and urinary bladder (16.7 at 8h post-dose).

Table 80. Radioactivity in Blood and Tissue Following 5 mg/kg Empagliflozin (BI 10773 XX) at Gestation Day 13 in the Rat

	ng Equivalents [^{14}C]-BI 10773/g				
	Animal Number (Sacrifice Time)				
Matrix	B13260 (1 Hour)	B13261 (2 Hours)	B13262 (4 Hours)	B13263 (8 Hours)	B13264 (24 Hours)
Adrenal gland	415	682	332	ND	ND
Amniotic fluid	BLQ	BLQ	BLQ	BLQ	ND
Amniotic sac	169	317	182	387	133
Bile	12600	13200	14500	2990	ND
Blood	219	377	155	60.5	ND
Bone	BLQ	BLQ	BLQ	BLQ	ND
Bone marrow	88.2	176	89.0	BLQ	ND
Cecum	173	1960	2210	914	694
Cecum contents	54.8	41100	49900	87000	3530
Cerebellum	BLQ	BLQ	BLQ	ND	ND
Cerebrum	BLQ	BLQ	BLQ	ND	ND
Diaphragm	ND	331	159	BLQ	ND
Esophageal contents	32800	42000	BLQ	5450	ND
Esophagus	414	713	299	224	ND
Exorbital lacrimal gland	153	257	144	60.3	ND
Eye	BLQ	BLQ	BLQ	BLQ	ND
Eye (lens)	BLQ	BLQ	BLQ	BLQ	ND
Fat (abdominal)	BLQ	BLQ	BLQ	BLQ	ND
Fat (brown)	137	226	137	ND	ND
Fetus	BLQ	BLQ	BLQ	BLQ	BLQ
Harderian gland	134	335	137	63.6	ND
Intra-orbital lacrimal gland	93.2	260	126	ND	ND
Kidney	2470	3670	2640	1610	442
Large intestinal contents	BLQ	BLQ	9410	BLQ	11800
Large intestine	ND	380	228	ND	ND
Liver	3840	4270	3060	1060	187
Lung	160	339	168	ND	ND
Lymph nodes	ND	282	ND	ND	ND
Mammary tissue	110	134	59.2	BLQ	ND
Medulla	BLQ	BLQ	BLQ	ND	ND
Muscle	82.2	203	104	BLQ	ND
Myocardium	208	439	172	ND	ND
Nasal turbinates	BLQ	69.5	BLQ	ND	ND
Olfactory lobe	BLQ	BLQ	BLQ	ND	ND
Ovary	75.7	208	181	BLQ	ND
Pancreas	273	528	290	83.6	ND
Pituitary gland	208	300	104	BLQ	ND
Placenta	127	178	150	59.6	ND
Preputial gland	142	271	106	BLQ	BLQ
Renal cortex	2960	3990	2800	2070	626

BLQ Below the limit of quantitation (<54.3 ng equivalents [^{14}C]-BI 10773/g).
ND Not detectable (sample shape not discernible from background or surrounding tissue).

Table 80. Continued

Matrix	ng Equivalents [^{14}C]-BI 10773/g Animal Number (Sacrifice Time) B13260 (1 Hour)	B13261 (2 Hours)	B13262 (4 Hours)	B13263 (8 Hours)	B13264 (24 Hours)
Renal medulla	1860	1850	1280	687	200
Salivary gland	181	357	204	80.7	ND
Skin	86.0	194	80.4	BLQ	ND
Small intestinal contents	50400	72200	105000	26200	122
Small intestine	266	3170	2900	2050	77.8
Spinal cord	BLQ	BLQ	BLQ	ND	ND
Spleen	136	210	123	BLQ	ND
Stomach	559	409	330	59.2	BLQ
Stomach contents	137000	107000	57000	53600	BLQ
Thymus	83.9	207	95.6	ND	ND
Thyroid	162	335	ND	ND	ND
Urinary bladder	139	332	88.6	1170	104
Urine	4070	15400	3560	19900	BLQ
Uterus	108	271	165	BLQ	BLQ
Uveal tract	69.3	182	86.7	65.4	ND
Vagina	110	227	130	ND	ND

BLQ Below the limit of quantitation (<54.3 ng equivalents [^{14}C]-BI 10773/g).
ND Not detectable (sample shape not discernible from background or surrounding tissue).

Group 2 Gestation Day 18

Fetal Tissue

- Radioactivity was not present in the amniotic fluid but was present at a low level in the fetus at 4 hours post-dose, showing that empagliflozin (or metabolites) was able to cross the placenta following a single dose.

Maternal Tissue

- Placental tissue contained empagliflozin at all time points examined.

- The peak concentration of radioactivity in the blood and plasma was 274 and 416 ng equivalents of [^{14}C]-empagliflozin/g (608 and 922 nM), respectively at 2 hours post-dose, and were quantifiable up to 24 hours post-dose.

- The blood: plasma concentration ranged from 0.641-0.714, suggesting empagliflozin and its metabolites were more associated with plasma rather than the blood and its components.

- The distribution of [^{14}C]-empagliflozin was limited with radioactivity quantifiable in each tissue, but not all time points, with the exception of bone, cerebellum, cerebrum, eye, eye lens, abdominal fat, lymph nodes, medulla, nasal turbinates, olfactory lobe, spinal cord and thyroid where no radioactivity was found.

- The highest concentration of radioactivity was found in the stomach contents and the small intestine contents consistent with oral dose administration.

- The tissue: plasma concentration ratio was less than one for most tissues; with high tissue: plasma concentration in the bile (≥20), cecum (≥4), liver (≥7), kidney (cortex and medulla) (≥6) and small intestine (≥3).

Table 81. Radioactivity in Blood and Tissues Following 5 mg/kg Empagliflozin (BI 10773 XX) at Gestation Day 18 in the Rat

	ng Equivalents [^{14}C]-BI 10773/g				
	Animal Number (Sacrifice Time)				
Matrix	B13265 (1 Hour)	B13266 (2 Hours)	B13267 (4 Hours)	B13268 (8 Hours)	B13269 (24 Hours)
Adrenal gland	587	712	317	169	ND
Amniotic fluid	BLQ	BLQ	BLQ	BLQ	ND
Amniotic sac	191	265	451	466	139
Bile	9750	11300	19100	3070	ND
Blood	208	286	168	80.0	ND
Bone	BLQ	BLQ	BLQ	BLQ	ND
Bone marrow	99.1	136	96.6	BLQ	ND
Cecum	145	1710	1700	1070	281
Cecum contents	141	8740	62600	79700	6710
Cerebellum	BLQ	BLQ	BLQ	BLQ	ND
Cerebrum	BLQ	BLQ	BLQ	BLQ	ND
Diaphragm	192	230	151	96.7	ND
Esophageal contents	60500	69300	112	176	74.3
Esophagus	178	1630	217	162	BLQ
Exorbital lacrimal gland	176	167	143	99.6	ND
Eye	BLQ	BLQ	BLQ	BLQ	ND
Eye (lens)	BLQ	BLQ	BLQ	BLQ	ND
Fat (abdominal)	BLQ	BLQ	BLQ	BLQ	ND
Fat (brown)	158	171	122	72.8	ND
Fetus	BLQ	BLQ	51.3	BLQ	BLQ
Harderian gland	110	152	186	109	ND
Intra-orbital lacrimal gland	126	151	ND	ND	ND
Kidney	2620	2720	2810	2130	606
Large intestinal contents	136	BLQ	50400	36600	6430
Large intestine	135	128	601	1130	171
Liver	2290	4280	2190	1310	145
Lung	191	212	178	81.6	ND
Lymph nodes	ND	ND	ND	ND	ND
Mammary tissue	93.9	115	86.1	81.8	ND
Medulla	BLQ	BLQ	BLQ	BLQ	ND
Muscle	78.6	113	119	71.6	ND
Myocardium	225	314	220	110	ND
Nasal turbinates	ND	ND	ND	BLQ	ND
Olfactory lobe	ND	ND	ND	BLQ	ND
Ovary	64.2	68.8	166	51.4	ND
Pancreas	297	437	251	181	ND
Pituitary gland	189	185	168	71.5	ND
Placenta	141	159	129	58.4	ND
Preputial gland	114	130	158	BLQ	ND
Renal cortex	3320	3250	2950	2560	918

BLQ Below the limit of quantitation (<50.7 ng equivalents [^{14}C]-BI 10773/g).
ND Not detectable (sample shape not discernible from background or surrounding tissue).

	ng Equivalents [^{14}C]-BI 10773/g				
	Animal Number (Sacrifice Time)				
Matrix	B13265 (1 Hour)	B13266 (2 Hours)	B13267 (4 Hours)	B13268 (8 Hours)	B13269 (24 Hours)
Renal medulla	1760	2080	2260	937	277
Salivary gland	196	239	217	138	ND
Skin	91.0	103	113	BLQ	ND
Small intestinal contents	24000	62500	159000	114000	768
Small intestine	1060	6130	2760	2250	308
Spinal cord	BLQ	ND	ND	ND	ND
Spleen	163	192	ND	ND	ND
Stomach	775	712	223	92.4	BLQ
Stomach contents	180000	143000	19200	5360	145
Thymus	120	161	ND	ND	ND
Thyroid	ND	ND	ND	ND	ND
Urinary bladder	116	824	275	66.4	NR
Urine	NR	12300	45900	1870	NR
Uterus	164	255	132	126	ND
Uveal tract	72.3	118	95.4	BLQ	ND
Vagina	145	131	149	ND	ND

BLQ Below the limit of quantitation (<50.7 ng equivalents [^{14}C]-BI 10773/g).
ND Not detectable (sample shape not discernible from background or surrounding tissue).
NR Not represented (tissue not present in section).

Group 3 Postnatal Day 11 and 12

- In blood, plasma and milk [^{14}C]-empagliflozin radioactivity reached C_{max} at 2 hours post-dose (see sponsor's table below)

- The mean maximal concentration for blood, plasma and milk were 664, 1020 and 1440 nM (300, 459 and 647 ng [^{14}C]-empagliflozin equivalents/g).

- The blood: plasma concentration ratios were less than one at all time points (see sponsor's table below).

- The mean milk to plasma ratio ranged from 0.634 -5 and was greater than 1 from 2 to 24 hours post-dose (see sponsor's table below).

- The mean maximal milk to plasma ratio of 5 occurred at 8 hours post-dose, suggesting accumulation of empagliflozin in the milk (see sponsor's table below).

Table 82. Blood, Plasma and Milk Concentration in Post-partum Days 11-12 Rats Exposed to 5 mg/kg [^{14}C]-Empagliflozin (BI 10773 XX)

Collection Time Point (Hours)	Concentrations of Radioactivity: Animal Number 1	2	3	Mean	SD
			nM		
			Blood		
1[b]	522	691	740	651	115
2[c]	359	931	702	664	288
4[d]	328	161	394	294	120
8[e]	41.8	169	66.9	92.5	67.2
24[f]	BLQ	6.86	7.76	4.87	4.25
			Plasma		
1[b]	832	1070	1140	1010	160
2[c]	545	1420	1090	1020	440
4[d]	497	225	591	437	190
8[e]	63.8	259	98.0	140	104
24[f]	8.54	10.7	10.7	10.0	1.3
			Milk		
1[b]	412	756	796	655	211
2[c]	1110	1880	1330	1440	400
4[d]	1190	1060	1370	1210	160
8[e]	310	505	803	539	248
24[f]	7.89	7.51	16.7	10.7	5.2
			ng equivalents [^{14}C]-BI 10773/g		
			Blood		
1[b]	235	312	334	294	52
2[c]	162	420	317	300	130
4[d]	148	72.7	178	133	54
8[e]	18.8	76.1	30.2	41.7	30.3
24[f]	BLQ	3.09	3.50	2.20	1.91
			Plasma		
1[b]	375	483	512	457	72
2[c]	246	641	490	459	199
4[d]	224	101	266	197	86
8[e]	28.8	117	44.2	63.3	47.1
24[f]	3.85	4.84	4.84	4.51	0.57

Collection Time Point (Hours)	Concentrations of Radioactivity Animal Number 1	2	3	Mean	SD
	ng equivalents [^{14}C]-BI 10773/g Milk				
1[b]	186	341	359	295	95
2[c]	499	846	598	647	179
4[d]	535	480	619	545	70
8[e]	140	228	362	243	112
24[f]	3.56	3.38	7.53	4.82	2.34

BLQ Below the limit of quantitation.
SD Standard deviation.

Table 83. Blood: Plasma and Milk: Plasma Concentration Ratios Following 5 mg/kg [^{14}C]-BI 10773 XX in the Female Lactating Rat

Collection Time Point (Hours)	Concentration Ratios Animal Number 1	2	3	Mean	SD
	Blood:Plasma				
1[a]	0.627	0.645	0.652	0.641	0.013
2[b]	0.659	0.655	0.646	0.654	0.007
4[c]	0.659	0.718	0.667	0.681	0.032
8[d]	0.655	0.651	0.683	0.663	0.017
24[e]	NA	0.639	0.723	0.681	NA
	Milk:Plasma				
1[a]	0.495	0.705	0.701	0.634	0.120
2[b]	2.03	1.32	1.22	1.52	0.44
4[c]	2.39	4.74	2.32	3.15	1.38
8[d]	4.87	1.95	8.20	5.00	3.13
24[e]	0.924	0.699	1.55	1.06	0.44

NA Not applicable.
SD Standard deviation.

Local Tolerance:

Dermal studies:

- The potential effect of empagliflozin to cause dermal sensitivity was assessed in a lymph node assay in mice. Empagliflozin had no effect on this assay at concentrations up to 25%, thus empagliflozin is unlikely to cause dermal sensitization.
- The dermal irritation potential of empagliflozin was assessed in New Zealand White rabbits where empagliflozin (500 mg) paste in water was applied to shaved skin under occluded patch for 4 hours. The area was washed and clinical signs of dermal irritations (erythema and edema) were observed for 72 hours post-treatment. Empagliflozin did not appear to be irritating to shaved skin of rabbits suggesting that empagliflozin may not be a dermal irritant to humans.

Eye irritation
A small amount of empagliflozin (10 mg) was applied to the corneal surface of the anesthetized right eye of rabbits. The eye was rinsed 24 hours after test article instillation and was examined at 1, 24, 48 and 72 hours post-instillation and scored for corneal involvement, irritation in the iris and conjunctivae redness, chemosis and irritation. Under the conditions of this study, empagliflozin was not irritating to the eye in rabbits

11 Integrated Summary and Safety Evaluation

The proposed empagliflozin film-coated tablet was submitted in accordance with 21 USC 505(b)(1) for the treatment of type 2 diabetes mellitus (T2DM). Empagliflozin is the third in class SGLT2 inhibitor that has been submitted to the Agency for approval.

A comprehensive battery of nonclinical studies were conducted to support the development of empagliflozin for chronic use. All pivotal nonclinical studies were conducted using oral administration of the drug, which is the clinical exposure route, and in accordance with US FDA GLP regulations (21CFR58) as stated by the sponsor. Most nonclinical studies were reviewed in the course of drug development and are summarized in the NDA review.

Safety margins to expected human exposure were estimated using C_{max} = 687 nmol/L and $AUC_{0\text{-}24h,ss}$ = 4740 nmol.h/L plasma exposure in T2DM subjects at the proposed maximum recommended human dose (MRHD) of 25 mg empagliflozin.

Impurities and Degradants
In the drug substance impurities (b) (4) were identified in the drug substance at (b) (4) %. These impurities were qualified by their use in 4 and 13 week pivotal nonclinical studies in the rat and also by evaluation in an Ames assay and an in vitro micronucleus study in CHO cells which were negative.

Pharmacology
Empagliflozin (BI 10773 or Jardiance™) is a selective inhibitor of sodium glucose co-transporter (SGLT) 2. SGLT2 is selectively expressed in the kidney S1 proximal tubule and is responsible for the renal reabsorption of glucose. Inhibition of SGLT2 by empagliflozin results in the excretion of glucose thereby producing glucosuria. When evaluated in vitro, empagliflozin was a potent and selective inhibitor of human (h) SGLT2 with an IC_{50} of 1.3 nmol/L relative to the closely related hSGLT1 (IC_{50} of 6278 nmol/L) thus showing a selectivity of 4829-fold. The glucuronide metabolites of empagliflozin (BI 1026317, BI 1026319 or BI 1026318) were weak inhibitors of hSGLT2 with IC_{50} of approximately 1-1.5 μM.

In nonclinical models empagliflozin promoted glucose excretion, polyuria, sodium and chloride excretion and lowered plasma glucose in diabetic and non-diabetic animal models under conditions of hyperglycemia (oral glucose tolerance test).

Safety pharmacology assessment of cardiovascular, neurological, pulmonary, renal and gastrointestinal effects of empagliflozin did not identify significant liabilities of acute administration of drug. Although the results from the gastrointestinal safety pharmacology study suggests limited drug-drug interaction effects are likely to occur in humans, higher exposures and multiple dosing has led to gastrointestinal effects in rodents that are related to the off-target inhibition of the closely related SGLT1 transporter. These typically result in increased calcium absorption as an (off-target) effect of SGLT1 inhibition, which in turn can manifest as mineralization in multiple tissues; as was observed in the chronic rat (3, 6 and 24 month) and acute 2 week dog studies.

Absorption, Distribution, Metabolism and Excretion
An oral dose of empagliflozin was rapidly absorbed and is approximately 94% and 89% bioavailable in mice and dogs, respectively, but only 31% bioavailable in the rat. Empagliflozin, however, distributes rapidly to most rat tissues with low amounts distributing to brain, spinal cord, bone, bone marrow, eye, eye lens, testis and uveal tract.

Empagliflozin has a longer half-life in humans (13 hours) than that in the mouse, rat, or dog (4-7 hours), suggestive of differential rates of renal elimination. Plasma protein binding was high in the nonclinical species (88-91%) but slightly lower in humans (84%). Partitioning of empagliflozin into blood cells was moderate in humans and in the nonclinical species (0.253 – 0.301 blood cell: plasma concentration ratio) suggesting the majority of empagliflozin remains in the plasma. As the concentration of empagliflozin in the plasma is qualitatively similar to that in the blood, blood clearance will approximate plasma clearance.

Empagliflozin demonstrated low (apical to basolateral) absorptive transport but high efflux (basolateral to apical) transport via P-gp and BCRP transporters in vitro, but also did not inhibit P-gp- or BCRP-mediated transport of probe substrates in Caco-2 and MDCK-MDR1 cell models. Empagliflozin was also a substrate for multiple human transporters OAT3, OATP1B1 and OATP1B3, but not OAT1 and OCT2 when stably transfected in kidney HEK293 cells. As an inhibitor in this cell line, empagliflozin inhibited human OAT3, OATP1B1, OATP1B3 and OAT2B1 with an IC_{50} in the range of 45-295 μM, but not OAT1 or OCT2 (IC_{50} greater than 1 mM). In contrast to Caco-2 and MDCK-MDR1 cells, empagliflozin inhibited BCRP- and MRP2 transport of probe substrates with an IC_{50} of 114 and 1399 μM, respectively. At the therapeutic maximum (25 mg) the C_{max} of empagliflozin is 687 nmol/L, suggesting minimal drug-drug interactions with these transporters.

With in vitro hepatocyte preparations, empagliflozin was found not to induce human hepatocyte CYP1A2, 2B or 3A4 mRNA or enzyme activity against CYP450 isozyme-specific substrates. In vitro metabolism studies with hepatocytes or microsomes from the rat, dog and humans, show empagliflozin metabolism is the order rat > dog >> humans, suggesting some resistance to in vitro metabolism in humans relative to the dog and the rat. This is consistent with a human volunteer radiolabel study (#1245.8) with a single p.o. [^{14}C]empagliflozin at 50 mg, which showed the majority of plasma empagliflozin is the unchanged parent (approx. 75%) and 54% and 41% of the dose was excreted in the urine and feces, respectively.

Analysis of the plasma, feces and urine from this human study (metabolism study# DM-08-1139, U09-3362-01) showed the most abundant plasma metabolites were three empagliflozin glucuronides (M626/1 (CD00006135), M626/2 (CD00006134) and M626/3 (CD00006136) representing 3.3-7.4% of the plasma radioactivity. Unchanged empagliflozin also represented 83% and 44% of the fecal and urinary radioactivity.

Consequently the potential for empagliflozin to inhibit UGT activity and the UGTs involved in the metabolism of empagliflozin was evaluated in human liver microsomes. The UGT isoforms involved in the formation of the empagliflozin glucuronides were identified as UGT1A3, 1A8, 1A9 and 2B7. Empagliflozin inhibited UGT1A1 activity with an IC_{50} of greater than 50 μM and a Ki of 25 μM. As the human C_{max} for empagliflozin is 0.687 μM at 25 mg (MRHD), UGT1A1 is unlikely to be inhibited by empagliflozin in vivo.

Further in vitro metabolism studies with human and nonclinical species hepatocytes showed four metabolites that were also observed as metabolites in human plasma. In contrast, ten empagliflozin metabolites were identified in the plasma from in vivo nonclinical studies in mice, rats and dogs. This suggests a more complex metabolic process in vivo. However no unique metabolites were identified in the nonclinical species or human plasma, with the exception of metabolite M625_4, which is a glucuronide product detected in trace amounts in concentrated human plasma. The ten identified metabolites consist of glucuronide and oxidation products of empagliflozin.

As a percentage of the plasma profile, empagliflozin was the predominant component in the plasma of the mouse (36-87%), rat (63-86%), dog (67-89%) and humans (76%). In vivo metabolite profiles were qualitatively similar in all species tested and there were no unique human metabolites. However, empagliflozin metabolism in humans occurs primarily via glucuronidation and this differs from the nonclinical species where empagliflozin is predominantly metabolized via oxidation reactions.

In follow up in vitro mechanistic studies, using kidney and liver microsomes from the mouse, rat and humans, the sponsor identified a new oxidative metabolite M466/2 and an unidentified aldehyde intermediate, that occurred predominantly in the male mouse kidney, and to a much lesser extent in the female mouse kidney, mouse liver, rat kidney and rat liver. Metabolite M466/2 was also not found in human kidneys or human liver following exposure to empagliflozin in vitro.

In addition, in the mouse carcinogenicity study, renal tumor formation was associated with degenerative tubular changes and this possibly could be due to the aldehyde intermediate metabolite. The sponsor proposes metabolite M466/2 and its breakdown products as key components in the mechanism of tumor formation in male mice at high doses. Due to the late submission of the mechanistic data to the NDA in the review cycle, the plausibility of the sponsor's proposal will be determined in a supplement to the NDA.

Furthermore, in the dog, nephritis and nephropathy were identified at high exposure multiples in nonclinical studies ranging from 2 weeks to 12 months, except in the 26-week dog study (see General Toxicology section below). Nephritis and nephropathy occurred in these dog studies without a defined mechanism and could be mediated by an unstable aldehyde metabolite (described above for the mouse) or other as yet, unidentified reactive intermediates. As oxidative metabolism is a minor pathway for empagliflozin in humans, it is unlikely to produce renal toxicity in humans.

Excretion of a single oral dose of [^{14}C]empagliflozin detected as drug-related activity was recovered in predominantly in the feces in the mouse, rat and dog (61 - 82%) followed by the urine (4 – 30%). Total recovery of radioactivity was 95-96%, 72-83% and 87-88% in the mouse, rat and dog, respectively. Empagliflozin was the major component of the feces in the species tested, except in the dog.

General Toxicology

Pivotal repeat dose studies were conducted in the Wistar (Han) rat and Beagle dogs up to 6 and 12 months duration, respectively.

In the 6 month pivotal rat study, rats were exposed to empagliflozin at 2-78x MRHD. Per the expected pharmacology, empagliflozin dose-dependently increased urinary glucose excretion which in turn also caused secondary pharmacological effects of polyuria and weight loss. Secondary pharmacological action of SGLT2 inhibition that resulted in polyuria may also have caused cortical tubule dilatation as was noted in both male and female rats at 700 mg/kg (35-78x MRHD) and in the 100 mg/kg females (19x MRHD).

In addition, a dose-dependent increase and exacerbation of kidney tubular and cortical mineralization was also noted in all treatment groups. The off-target effect of tissue mineralization is likely due to modulation of calcium homeostasis due increased urinary calcium excretion and a compensatory increase in calcium absorption, that leads to tissue mineralization as a down stream event of excess calcium.

In the 52 week dog study, treated dogs were exposed to empagliflozin at 17-261x MRHD. A dose related increase in severity of vacuolation of the adrenal (zona glomerulosa) was observed. Adrenal vacuolation likely occurred due to osmotic and/or diuretic effect of enhanced glucose excretion (e.g. polyuria) and a resultant increase in aldosterone production due to known enhanced sodium excretion as a result of SGLT2

inhibition. Pharmacodynamic action also resulted in the expected reduced body weight, due to caloric loss of glucose (which was also observed in the 2-26 week dog studies).

Nephritis and cortical tubular degeneration with fibrosis was also observed in the high dose animals (219 and 261x MRHD in males and females respectively). Interstitial nephritis was also observed in the 2, 4, and 13 weeks dog studies, mostly in high dose animals at exposures of 152-358x MRHD. Nephritis and nephropathy was however, not observed in the 6 month dog study. Exposure to empagliflozin in the 6 month dog study was only136-146x MRHD and nephrotoxicity only occurred in dogs with higher exposures in the 13 week dog study (217-289x MRHD) and in the 52 week dog study (219-261x MRHD), respectively. This exposure difference may explain the lack of findings in the 6 month dog study

Overall, target organ toxicities in adult dogs occurred at high exposure multiples (≥152x MRHD) and the safety margins to the final clinical dose are high, suggesting low clinical risk of similar findings in humans.

A summary of the sub-chronic and chronic toxicity studies is given in the table below.

Reproductive Toxicology
Reproductive and developmental toxicity were assessed in fertility, early embryonic development and pre- and post-natal development animal studies.

In the fertility study in rats, mortality of unknown cause was observed in one 700 mg/kg male (155x MRHD). Paternal toxicity in the form of reduced body weight gain was observed in the pre-mating period (mid and high dose males) and also at the end of the study (all empagliflozin-treated males). Body weight gain and food consumption was also reduced in the 700 mg/kg females (155x MRHD). Consequently, the paternal and maternal systemic NOAEL was 300 mg/kg (48x MRHD). However, as there were no effects on fertility or reproductive performance in males and females the NOAEL for fertility is 700 mg/kg or 155x MRHD.

In a rat embryo-fetal development study empagliflozin was not teratogenic at 300 mg/kg (48x MRHD). Higher exposure resulted in a skeletal malformation of bent limb bone in the 700 mg/kg fetuses (154x MRHD). Maternal toxicity of reduced body weight, body weight gain and food consumption was observed in the 300 and 700 mg/kg dams, resulting in a NOAEL for maternal toxicity of 100 mg/kg (22x MRHD). Empagliflozin was also not teratogenic at 300 mg/kg in a rabbit embryo-fetal development study (128x MRHD). Higher exposure at 700 mg/kg (139x MRHD) resulted in abortions in 3 female rabbits that was likely due to reduced body weight gain during treatment. Two of the three aborting females also had completely resorbed litters. Consequently the percentage per litter post-implantation loss was increased in the 700 mg/kg fetuses, resulting in a reduction of the number of viable fetuses. Due to the abortions and reduced body weight gain the NOAEL for maternal toxicity in rabbits was 300 mg/kg (128x MRHD).

Cross-species concordance for maternal toxicity due to reduced body weight, suggest a potential risk in humans. However, the maternal toxicity occurred at high exposure multiples in both the rat (48x MRHD) and rabbit (139x), thus reducing the clinical risk. Empagliflozin was also not teratogenic at 48x and 128x MRHD in the rat and rabbit, respectively. The limited findings and the high safety margins suggest empagliflozin is unlikely to be teratogenic in humans at the highest clinical dose.

Exposure to empagliflozin at 100, 300 or 700 mg/kg in a pre- and postnatal development study in the rat, resulted in reduced body weight gain in the 300 and 700 mg dams during gestation (GD 6-20). The NOAEL for maternal toxicity is considered 100 mg/kg. For the pups, body weight/ body weight gain was dose-dependently reduced during the lactation period. The physical exam also noted "small stature" of the 700 mg/kg pups. Due to the significantly reduced body weight gain (up to 45%) in the empagliflozin-treated pups the sponsor terminated the between PND 19-24. The significant weight loss in pups is clear evidence of drug exposure in the milk resulting in an adverse outcome. The sponsor did not evaluate empagliflozin in the present study but carried out another pre- and postnatal study at lower doses of 0, 10, 30 and 100 mg/kg, a tissue distribution study in pregnant rats and a rat lacteal excretion study.

Exposure to empagliflozin at 10, 30 or 100 mg/kg in a pre- and postnatal development study did not result in maternal toxicity and the NOAEL for maternal toxicity is 100 mg/kg (16x MRHD). Dose-dependent reduced body weight and body weight gain was observed in the F_1 pups, particularly in HD males during weaning. The F_1 males also had a deficit in learning and memory at 100 mg/kg at PND 22 but not at PND 62. No effects were observed for the F_1 mating and reproductive performance and there were no morphological changes in the F_2 pups. The NOAEL for F_1 reproductive toxicity and F_2 toxicity was 100 mg/kg (16x MRHD). The NOAEL for F_1 toxicity was 30 mg/kg which is 4x MRHD.

Lowering the exposure to empagliflozin in the second pre- and postnatal study negated the maternal toxicity observed in the initial study, due to a lack of reduced body weight/body weight gain. However, reduced body weight/body weight gain were observed in the pups during weaning and this is likely due to lactational exposure to empagliflozin (as confirmed in the milk distribution study (see below)). The deficit in memory and learning was also likely due to failure of the rat pups to gain weight and was not observed in the older animals. Distribution of empagliflozin has not been determined in human milk and due to a potential clinical developmental risk of reduced weight or body weight gain in humans, nursing or exposure to empagliflozin should be discontinued in nursing mothers.

In a distribution study pregnant rats were treated with a single dose [^{14}C]empagliflozin at GD 13 or 18 for tissue distribution or PND 11 and 12 for milk and blood distribution. In pregnant rats, the blood, plasma and milk distribution reached C_{max} at 2 hours post-dose. The mean milk to plasma ratio ranged from 0.634 -5, and was greater than 1 from 2 to 24 hours post-dose. The mean maximal milk to plasma ratio of 5 occurred at 8 hours post-dose, suggesting accumulation of empagliflozin in the milk.

The distribution of [^{14}C]empagliflozin was limited with radioactivity quantifiable in each tissue, but not at all time points. However, at GD 18 a low amount of radioactivity was found in the fetus at 4 hours post-dose, showing that empagliflozin or a metabolite was able to cross the placenta. Of note, the sponsor used a low 5mg/kg single dose for this study, compared to the 100 to 300 mg/kg multiple doses used in segment II and III studies. This study likely underestimates fetal transfer of drug because the sponsor only tested a low, single oral dose of empagliflozin.

Distribution of empagliflozin in the rat milk constitutes a human clinical risk and necessitates discontinuation of nursing or exposure to empagliflozin in nursing mothers. The presence of empagliflozin in the fetus, albeit at very low amounts, shows empagliflozin or a metabolite is likely to cross the placenta in humans. However, as empagliflozin is not teratogenic in the rat or rabbit at 48x or 128x the 25 mg clinical dose, respectively, the likelihood of an adverse out come due to teratogencity in humans is unlikely.

Standard reproductive toxicology studies with some other SGLT2 inhibitors have reported morphological effects in the kidneys (dilatation of renal tubules and pelvi) in juvenile rats and are considered secondary to the pharmacological action of SGLT2 inhibition. Due to differences in timing of kidney development/maturation between rats and humans, these adverse effects seen in the kidneys of juvenile rats are considered relevant to the assessment of reproductive and developmental risk. The substantial difference in the toxicity profile for effects on the kidney between the standard reproductive toxicology studies and juvenile animal studies may reflect exposure to the test-article during a 'critical window' of renal development. At the present time, juvenile toxicity studies in the rat for empagliflozin were not conducted by the sponsor. However, as empagliflozin was present in fetal tissues and in the maternal milk it presents a potential developmental risk in the second/third trimesters of pregnancy and during nursing. The presence of an SGLT2 inhibitor in fetal tissues and/or in maternal milk of rats is considered sufficient evidence of potential human risk, which would be conveyed in drug labeling.

Genetic Toxicology

Empagliflozin was not mutagenic or clastogenic in an in vitro Ames assays, an in vitro mouse lymphoma L5178Y tk+/- assay or in vivo assays: rat blood cell micronucleus assay and a rat bone marrow micronucleus assay. All metabolites of empagliflozin in human subjects have been indentified in mice and rats in vivo, and would have been evaluated for genotoxic potential in these studies. The weight of evidence suggests that empagliflozin and its identified metabolites are unlikely to be mutagenic or clastogenic in human subjects.

Carcinogenicity

Empagliflozin was assessed for its potential to induce tumors in two-year bioassays conducted in rats and mice. The two-year bioassays are intended to detect drug-

induced tumors that arise from genotoxic as well as non-genotoxic mechanisms of action after approximately life-time exposure to an investigational drug. Empagliflozin doses used in the carcinogenicity studies provided greater than 25-fold multiples of human exposure for both species and treatment regimens and final reports were considered appropriate by the Division and the Executive Carcinogenicity Assessment Committee (ECAC).

In rats, life time exposure to empagliflozin did not increase the incidence of tumors in females at drug exposures reaching 72x the clinical dose (25 mg). In male rats, empagliflozin dose-dependently increased the incidence of whole body/cavity hemangioma which became significant at 700 mg/kg (high dose) which is 42x MRHD. The increase was nearly entirely due to a higher incidence of hemangioma in the mesenteric lymph nodes. Empagliflozin also numerically increased testicular Leydig cell tumors in the 300 and 700 mg/kg males and this increase is likely to be related to drug treatment and is consistent with the results of several SGLT2 inhibitors. Leydig tumor formation in humans is rare and unlikely to be encountered in humans due to the known physiological differences between the rat and human for this tumor. Treatment with empagliflozin at 300 and 700 mg/kg in male rats is equivalent to 26x and 42x MRHD.

Empagliflozin also had minimal impact on survival in rats and body weight was dose-dependently reduced in all treated animals. Extensive cortical tubule dilatation was observed in all empagliflozin-treated animals particularly males. Mineralization was observed in multiple tissues including the kidney and heart. Bone accretion was observed in the 300 and 700 mg/kg males; and a high incidence of residual cartilage of the diaphysis was increased in the femur of all empagliflozin-treated animals particularly at 300 and 700 mg/kg. Liver vacuolation of sinusoidal cells was dose-dependently increased in all empagliflozin-treated males and females. The NOAEL in the rat study was 100 mg/kg which is 17-21x MRHD.

In mice, empagliflozin did not increase drug-related neoplasms in female mice at up to 62x MRHD. Renal tubular adenoma and carcinoma (combined) were significantly increased in the 1000 mg/kg males and was accompanied by a high incidence of renal atypical hyperplasia in the same group. Renal cystic tubular hyperplasia increased at all doses in the empagliflozin-treated animals, particularly the males. The renal neoplasms also occurred in the presence of renal tubular injury (single cell necrosis, karyomegaly, hypertrophy, atrophy, cysts and pelvic dilatation) in the dosed groups. Treatment with empagliflozin at 1000 mg/kg in male mice is equivalent to 45x MRHD.

Decreased survival was observed in the 1000 mg/kg males that resulted in early termination of this group at week 97. Increased mortality in the HD male group was due to urinary tract dilatation. Chronic progressive nephropathy (CPN) was present in all mice but the severity was elevated in the 1000 mg/kg males. The incidence and severity of ureter dilatation and the incidence of urinary bladder dilatation was also exacerbated in the empagliflozin-treated males. Hepatic cytoplasmic vacuolation was increased in all animals at ≥ 300 mg/kg. Exposure margins at 300 mg/kg were 11x and

28x MRHD in males and females respectively. The NOAEL in the mouse study was 100 mg/kg which is 4-7x MRHD.

Overall, empagliflozin poses minimal carcinogenic risk to humans based on the high exposure multiples that caused tumor formation in a single sex in mice (x45 MRHD) and rats (42x MRHD), respectively, and the high exposure multiples at the NOAEL in the rat (17-21x MRHD) and the mouse (4-7x MRHD).

Special Toxicology Studies
Dermal and Eye Irritation Studies
Empagliflozin had no effect on dermal sensitization at concentrations up to 25% in a local lymph node assay and did not cause dermal irritation in rabbits. Empagliflozin at 10 mg was not irritating to the eye in rabbits.

Table 84. Summary of Sub-Chronic and Chronic Toxicology Studies.

SPECIES TOXICOLOGY STUDIES			
SPECIES/ STUDY	**NOAEL**	**MULTIPLE OF MRHD 25 mg: 4670 nmol.hr/L AUC basis***	**BASIS**
Mouse 13 weeks: 0, 500, 750 and 1000 mg/kg/day	1000 mg/kg/day	M: 62x F: 98x	-Mortality at 250 and 400 mg/kg. -Increased prostatic weight (≥20%) at 150 and 250 mg/kg.
Rat 13 weeks: 0, 30, 100 and 700 mg/kg/day	100 mg/kg/day	M: 13x F: 35x	-Mortality at 700 mg/kg. -Increased BUN at 100 mg/kg without pathology correlate. -Dose-related mineralization in the kidney.
Rat 6 month: 0, 30, 100 and 700 mg/kg/day	Not established	M: NA F: NA	-Mortality 1M, 1F at 700 mg/kg -All empagliflozin treatment groups: Adrenal gland vacuolation, Hepatic vacuolation, Kidney corticomedullary tubular dilatation and mineralization. -Kidney vacuolation at ≥ 100 mg/kg
Dog 13 weeks: 0, 10, 30 and 100 mg/kg/day	10 mg/kg/day	M: 15x F: 24x	-Nephritis and cortical tubular nephropathy at 30 mg/kg that irreversible at 100 mg/kg -Hepatic vacuolation at 30 mg/kg
Dog 26 weeks: 0, 10, 30 and 100 mg/kg/day	10 mg/kg/day	M: 15x F: 12x	-Reduced BW/BW gain at 30 and 100 mg/kg -Hepatic vacuolation, degeneration and Kupffer cell hypertrophy at 100 mg/kg
Dog 52 weeks: 0, 10, 30 and 100 mg/kg/day	30 mg/kg/day	M: 55x F: 50x	-Mortality at 30 mg/kg. -Nephritis and cortical tubular degeneration at 100 mg/kg -Dose-dependent reduced BW. -Adrenal gland vacuolation at 30 and 100 mg/kg

*AUC in human: 4740 nmol.hr/L at 25 mg/day, NA – not applicable

12 Appendix/Attachments

Appendix A: Screening Panel Data for Empagliflozin Binding Activity against various Receptors, Ion Channels, Proteases and Transporters

Cat. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION %	IC_{50}	K_i	n_H	R
200510	Adenosine A_1	133848	hum	2	10 µM	14				
200610	Adenosine A_{2A}	133849	hum	2	10 µM	-4				
200720	Adenosine A_3	133887	hum	2	10 µM	5				
203100	Adrenergic α_{1A}	133985	rat	2	10 µM	3				
203200	Adrenergic α_{1B}	133986	rat	2	10 µM	-4				
203400	Adrenergic α_{1D}	133987	hum	2	10 µM	-4				
203620	Adrenergic α_{2A}	133851	hum	2	10 µM	1				
204010	Adrenergic β_1	133888	hum	2	10 µM	-7				
204110	Adrenergic β_2	133889	hum	2	10 µM	-1				
212510	Bradykinin B_1	133854	hum	2	10 µM	-8				
212610	Bradykinin B_2	133855	hum	2	10 µM	-6				
214510	Calcium Channel L-Type, Benzothiazepine	133958	rat	2	10 µM	5				
214600	Calcium Channel L-Type, Dihydropyridine	133984	rat	2	10 µM	12				
216000	Calcium Channel N-Type	134060	rat	2	10 µM	-11				
219500	Dopamine D_1	133856	hum	2	10 µM	-4				
219700	Dopamine D_{2S}	133857	hum	2	10 µM	10				
219800	Dopamine D_3	133858	hum	2	10 µM	-4				
219900	Dopamine $D_{4.2}$	133859	hum	2	10 µM	4				
224010	Endothelin ET_A	134086	hum	2	10 µM	9				
224110	Endothelin ET_B	134087	hum	2	10 µM	-3				
225500	Epidermal Growth Factor (EGF)	133876	hum	2	10 µM	-3				
226010	Estrogen ERα	133891	hum	2	10 µM	6				
226500	$GABA_A$, Agonist Site	133860	rat	2	10 µM	-5				
226600	$GABA_A$, Benzodiazepine, Central	133861	rat	2	10 µM	-3				
228600	$GABA_{BIA}$	134091	hum	2	10 µM	7				
232010	Glucocorticoid	133988	hum	2	10 µM	8				
232700	Glutamate, Kainate	133895	rat	2	10 µM	-6				
232810	Glutamate, NMDA, Agonism	133896	rat	2	10 µM	24				
232910	Glutamate, NMDA, Glycine	133862	rat	2	10 µM	-3				
233000	Glutamate, NMDA, Phencyclidine	133863	rat	2	10 µM	1				

* Batch: Represents compounds tested concurrently in the same assay(s). ‡ Partially soluble in *in vitro* test solvent.
♦ Denotes item meeting criteria for significance
† Results with ≥ 50% stimulation or inhibition are highlighted. (Negative values correspond to stimulation of binding or enzyme activity)
R=Additional Comments
ham=hamster; hum=human

Cat. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION						IC50	Ki	nH	R
						%	-100	-50	0	50	100				
239610	Histamine H_1	133898	hum	2	10 µM	0									
239710	Histamine H_2	133899	hum	2	10 µM	0									
239810	Histamine H_3	133970	hum	2	10 µM	8									
241000	Imidazoline I_2, Central	133971	rat	2	10 µM	9									
243510	Interleukin IL-1	134067	mouse	2	10 µM	1									
250460	Leukotriene, Cysteinyl $CysLT_1$	133900	hum	2	10 µM	-2									
251600	Melatonin MT_1	134088	hum	2	10 µM	-1									
252600	Muscarinic M_1	133962	hum	2	10 µM	-3									
252700	Muscarinic M_2	133963	hum	2	10 µM	0									
252800	Muscarinic M_3	133964	hum	2	10 µM	8									
257010	Neuropeptide Y Y_1	133902	hum	2	10 µM	-7									
257110	Neuropeptide Y Y_2	133903	hum	2	10 µM	4									
258590	Nicotinic Acetylcholine	133978	hum	2	10 µM	-5									
258700	Nicotinic Acetylcholine, Bungarotoxin-Sensitive, Neuromuscular	134083	hum	2	10 µM	-4									
260110	Opiate δ (OP1, DOP)	133974	hum	2	10 µM	9									
260210	Opiate κ (OP2, KOP)	133975	hum	2	10 µM	-5									
260410	Opiate µ (OP3, MOP)	133976	hum	2	10 µM	2									
264500	Phorbol Ester	133865	mouse	2	10 µM	-9									
265010	Platelet Activating Factor (PAF)	134093	hum	2	10 µM	2									
265600	Potassium Channel [K_{ATP}]	134085	ham	2	10 µM	4									
265900	Potassium Channel HERG	133866	hum	2	10 µM	0									
268410	Prostanoid EP_4	134004	hum	2	10 µM	0									
268700	Purinergic P_{2X}	134057	rabbit	2	10 µM	-5									
268810	Purinergic P_{2Y}	134058	rat	2	10 µM	3									
270000	Rolipram	133867	rat	2	10 µM	-4									
271110	Serotonin (5-Hydroxytryptamine) 5-HT_{1A}	133868	hum	2	10 µM	19									
271910	Serotonin (5-Hydroxytryptamine) 5-HT_3	133873	hum	2	10 µM	-2									
278110	Sigma σ_1	133960	hum	2	10 µM	-10									
278200	Sigma σ_2	133961	rat	2	10 µM	-2									
279510	Sodium Channel, Site 2	133875	rat	2	10 µM	-20									

* Batch: Represents compounds tested concurrently in the same assay(s). ‡ Partially soluble in *in vitro* test solvent.
♦ Denotes item meeting criteria for significance
† Results with ≥ 50% stimulation or inhibition are highlighted. (Negative values correspond to stimulation of binding or enzyme activity)
R=Additional Comments
ham=hamster; hum=human

Cat. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION	IC_{50}	K_i	n_H	R
						% -100 -50 0 50 100				
255510	Tachykinin NK_1	134194	hum	2	10 µM	9				
285010	Testosterone	133904	rat	2	10 µM	2				
285900	Thyroid Hormone	133820	rat	2	10 µM	19				
220320	Transporter, Dopamine (DAT)	133981	hum	2	10 µM	1				
226400	Transporter, GABA	133893	rat	2	10 µM	-7				
204410	Transporter, Norepinephrine (NET)	133980	hum	2	10 µM	-5				
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	133983	hum	2	10 µM	0				

Cat. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION	IC_{50}	K_i	n_H	R
						% -100 -50 0 50 100				
176200	Protein Serine/Threonine Kinase, IKK-1	141765	hum	2	10 µM	16				
171380	Protein Serine/Threonine Kinase, MAPKAPK5 (PRAK)	141767	hum	2	10 µM	-1				
175000	Protein Tyrosine Kinase, Insulin Receptor	141429	hum	2	10 µM	-1				

* Batch: Represents compounds tested concurrently in the same assay(s). ‡ Partially soluble in *in vitro* test solvent.
♦ Denotes item meeting criteria for significance
† Results with ≥ 50% stimulation or inhibition are highlighted. (Negative values correspond to stimulation of binding or enzyme activity)
R=Additional Comments
ham=hamster; hum=human

Cat. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION	IC_{50}	K_i	n_H	R
						% -100 -50 0 50 100				
107710	ATPase, Na^+/K^+, Heart, Pig	141781	pg	2	10 µM	-4				
112000	Carbonic Anhydrase	141857	hum	2	10 µM	2				
112100	Carnitine Palmitoyltransferase-1 (CPT-1)	141768	rat	2	10 µM	7				
112200	Catechol-O-Methyltransferase (COMT)	141770	pg	2	10 µM	27				
104010	Cholinesterase, Acetyl, ACES	141569	hum	2	10 µM	-1				
116020	Cyclooxygenase COX-1	141867	hum	2	10 µM	-4				
118050	CYP450, 1A2	141570	hum	2	10 µM	-1				
118070	CYP450, 2C19	141572	hum	2	10 µM	12				
118060	CYP450, 2C9	141571	hum	2	10 µM	7				
118080	CYP450, 2D6	141573	hum	2	10 µM	4				
118090	CYP450, 3A4	141574	hum	2	10 µM	6				
140010	Monoamine Oxidase MAO-A	141779	hum	2	10 µM	-1				
140120	Monoamine Oxidase MAO-B	141780	hum	2	10 µM	9				
143000	Nitric Oxide Synthase, Endothelial (eNOS)	141773	bov	2	10 µM	-6				
142000	Nitric Oxide Synthase, Neuronal (nNOS)	141772	rat	2	10 µM	0				
107300	Peptidase, Angiotensin Converting Enzyme	141701	rabbit	2	10 µM	1				
112350	Peptidase, CTSD (Cathepsin D)	141645	hum	2	10 µM	-1				
113500	Peptidase, Factor VIIa	141783	hum	2	10 µM	0				
113600	Peptidase, Factor Xa	141784	hum	2	10 µM	-5				
164000	Peptidase, Metalloproteinase, Neutral Endopeptidase	141778	hum	2	10 µM	-5				
165000	Peptidase, Thrombin	141771	hum	2	10 µM	0				
165050	Peptidase, Tissue Plasminogen Activator (tPA)	141776	hum	2	10 µM	1				
165100	Peptidase, Trypsin	141775	hum	2	10 µM	2				
152000	Phosphodiesterase PDE3	141787	hum	2	10 µM	16				
154000	Phosphodiesterase PDE4	141788	hum	2	10 µM	0				
156100	Phosphodiesterase PDE6	141790	bov	2	10 µM	11				
167000	Proteasome	141777	hum	2	10 µM	3				
168000	Protein Serine/Threonine Kinase, Ca^{2+}/Calmodulin-Dep. II	141766	rat	2	10 µM	-1				

* Batch: Represents compounds tested concurrently in the same assay(s). ‡ Partially soluble in *in vitro* test solvent.
♦ Denotes item meeting criteria for significance
† Results with ≥ 50% stimulation or inhibition are highlighted. (Negative values correspond to stimulation of binding or enzyme activity)
R=Additional Comments
bov=bovine; hum=human; pg=pig

Appendix B: Rat Biostatistics Review

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pair-wise Comparisons (each of vehicle controls or combined controls, low, medium and high dose groups)

Organ Name	Tumor Name	Vehicle Cont1 N 50	100 mg Low N 50	300 mg Med N 50	700 mg High N 50	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Male									
Body, Whole/Cav	B-Hemangioma	3	2	5	9	0.008	0.834	0.370	0.053
		[36]	[34]	[34]	[35]	.	.	.	.
	M-Histiocytic Sarcom	1	0	0	3	0.048	.	.	0.291
		[37]	[34]	[34]	[35]	.	.	.	.
	M-Lymphosarcoma	0	0	5	0	0.428	.	0.033	.
		[36]	[34]	[36]	[35]	.	.	.	.

Organ Name	Tumor Name	Vehicle Cont2 N 50	100 mg Low N 50	300 mg Med N 50	700 mg High N 50	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Male									
Body, Whole/Cav	B-Hemangioma	0	2	5	9	0.001	0.271	0.033	0.001
		[27]	[34]	[34]	[35]	.	.	.	.
	M-Histiocytic Sarcoma	0	0	0	3	0.018	.	.	0.125
		[27]	[34]	[34]	[35]	.	.	.	.
	M-Lymphosarcoma	0	0	5	0	0.491	.	0.037	.
		[27]	[34]	[36]	[35]	.	.	.	.
Testis	B-Interstitial Cell tumor	0	4	7	6	0.057	0.070	0.008	0.014
		[27]	[34]	[34]	[35]	.	.	.	.
Thyroid									
	B-Adenoma, Follicular	2	7	13	4	0.582	0.102	0.003	0.350
		[27]	[34]	[34]	[35]	.	.	.	.
	FOLLICULAR_CELL_ADEN NOMA+CARCINOMA	3	8	13	6	0.478	0.121	0.007	0.241
		[27]	[34]	[34]	[35]	.	.	.	.

Organ Name	Tumor Name	Combined Conts N 100	100 mg Low N 50	300 mg Med N 50	700 mg High N 50	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Male									
Body, Whole/Cav	B-Hemangioma	3	2	5	9	0.001	0.567	0.089	0.002
		[63]	[34]	[34]	[35]	.	.	.	.
	M-Histiocytic Sarcoma	1	0	0	3	0.030	.	.	0.105
		[63]	[34]	[34]	[35]	.	.	.	.
	M-Lymphosarcoma	0	0	5	0	0.294	.	0.005	.
		[63]	[34]	[36]	[35]	.	.	.	.

Testis	B-Interstitial Cell tumor	2	4	7	6	0.022	0.111	0.008	0.017
		[63]	[34]	[34]	[35]	.	.	.	.
Thyroid									
	B-Adenoma, Follicula	11	7	13	4	0.681	0.435	0.021	0.801
		[63]	[34]	[34]	[35]	.	.	.	.
	FOLLICULAR_CELL_ADEN MA+CARCINOMA	13	8	13	6	0.583	0.447	0.048	0.654
		[63]	[34]	[34]	[35]	.	.	.	.
Female									
Cervix									
	B-Polyp, Endometrial	0	3	0	1	0.416	0.044	.	0.336
		[67]	[39]	[35]	[33]	.	.	.	.

Appendix C: Mouse Biostatistics Review

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pair-wise Comparisons (Vehicle control 1, 2 & combined vehicle controls, low, medium and high dose groups)

					1000 m			
		Vehicle	100 mg	300 mg	g			
		Cont1	Low	Med	High	P_Value	P_Value	P_Value	P_Value
Organ Name	Tumor Name	N 50	N 50	N 50	N 50	Dos Resp	C vs. L	C vs. M	C vs. H
Male									
Kidney	B-Adenoma, Tubular	0	0	1	3	0.007	.	0.493	0.105
		[32]	[24]	[23]	[19]	.	.	.	.
	M-Carcinoma, Tubular	0	0	0	2	0.036	.	.	0.226
		[32]	[24]	[23]	[19]	.	.	.	.
	CARCINOMA+ADENOMA	0	0	1	5	< .0001	.	0.493	0.021
		[32]	[24]	[23]	[19]	.	.	.	.
Female									
Body, Whole/Cav									
	M-Histiocytic Sarcom	1	6	3	6	0.035	0.023	0.277	0.023
		[25]	[22]	[23]	[18]	.	.	.	.
Uterus									
	M-Carcinoma, Endomet	0	0	0	2	0.042	.	.	0.184
		[25]	[20]	[23]	[18]	.	.	.	.

					1000 m			
		Vehicle	100 mg	300 mg	g			
		Cont2	Low	Med	High	P_Value	P_Value	P_Value	P_Value
Organ Name	Tumor Name	N 50	N 50	N 50	N 50	Dos Resp	C vs. L	C vs. M	C vs. H
Male									
Kidney	B-Adenoma, Tubular	0	0	1	3	0.008	.	0.478	0.096
		[31]	[24]	[23]	[19]	.	.	.	.
	M-Carcinoma, Tubular	0	0	0	2	0.037	.	.	0.214
		[31]	[24]	[23]	[19]	.	.	.	.
	CARCINOMA+ADENOMA	0	0	1	5	<.0001	.	0.478	0.019
		[31]	[24]	[23]	[19]	.	.	.	.

Female										
	Uterus									
		M-Carcinoma, Endomet	0	0	0	2	0.044	.	.	0.237
			[23]	[20]	[23]	[18]	.	.	.	.

	Organ Name	Tumor Name	Combined Conts N 100	100 mg Low N 50	300 mg Med N 50	1000 mg High N 50	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Male										
	Kidney	B-Adenoma, Tubular	0	0	1	3	0.002	.	0.320	0.028
			[63]	[24]	[23]	[19]	.	.	.	.
		M-Carcinoma, Tubular	0	0	0	2	0.021	.	.	0.094
			[63]	[24]	[23]	[19]	.	.	.	.
		CARCINOMA+ADENOMA	0	0	1	5	<.0001	.	0.320	0.002
			[63]	[24]	[23]	[19]	.	.	.	.
Female										
	Uterus									
		M-Carcinoma, Endomet	0	0	0	2	0.026	.	.	0.087
			[48]	[20]	[23]	[18]	.	.	.	.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MUKESH SUMMAN
11/05/2013

TODD M BOURCIER
11/05/2013
Pharm/tox supports AP

Memorandum

PHARMACOLOGY/TOXICOLOGY MEMO TO FILE

Date:	28th October 2013
NDA #	204629
Sponsor:	Boehringer Ingelheim Pharmaceuticals Inc
Drug:	Empagliflozin, SGLT2 inhibitor
Reviewer:	Mukesh Summan, PhD DABT

Re: October 28th 2013 Teleconference with Boehringer Ingelheim Pharmaceuticals Inc.

The sponsor submitted a nonclinical summary document on October 18th of studies conducted to identify metabolites and (proposed) mode of action for the occurrence of renal tumors in male mice. A teleconference was held between the Agency and Sponsor as a consequence of a newly identified metabolite found in the nonclinical species and the discrepancies in the documentation that was provided.

The sponsor began the meeting and discussed the mode of action for the mouse renal tumor signal and its specificity to the male mouse and not other nonclinical species or humans. The sponsor then described the in vitro metabolism studies conducted with empagliflozin that result in formation of metabolite M466/2 and/or an unstable aldehyde metabolite, its subsequent (b) (4) and metabolite M380/1 or to other metabolites (M482/1, M482/2, M468/1 etc). Metabolite M466/2 was identified as the key metabolite found in the male mouse kidney via oxidative metabolism that the sponsor proposes is involved in tumor formation. The sponsor clarified that the male mouse specificity of M466/2 production is based solely on liver and microsome studies, and stated they have confidence that the results predict in vivo metabolism. The sponsor then described the human in vitro metabolic pathway, which is primarily via glucuronidation and that glucuronide metabolites were not found in vitro in the male and female kidneys in mice. The sponsor also described the breakdown products of the unstable aldehyde that are potential surrogates for identification of the unstable aldehyde metabolite.

Nonclinical concern arose as despite only primarily describing mouse and human data, it became evident that downstream metabolites of metabolite M466/2 and/or the unstable aldehyde were present in the plasma of multiple nonclinical species and also in human plasma and urine. In addition, nephritis and nephropathy have been described in non-clinical dog species at high doses in studies ranging from 2 weeks to 12 months, without a defined mechanism and could possibly be mediated by an unstable aldehyde.

From the subsequent discussion it became evident that it is likely that aldehyde metabolites are likely to be present in all nonclinical species and only the M466/2 metabolite has been identified in the mouse, thus far. In addition, the sponsor stated that similar mechanistic in vitro studies for

the dog had not been conducted. Consequently, the clinical risk in humans can be inferred by the known different degrees of oxidative versus conjugative metabolism between the species, the metabolites identified thus far in the in vivo ADME studies (including human studies) and the high exposure multiples required to produce nephritis/nephropathy in the dog (≥150x the 25 mg clinical dose).

Given the lateness of the submission, the sponsor was notified that the new metabolism information but not the mode-of-action information would be reviewed in this review cycle.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MUKESH SUMMAN
10/29/2013

TODD M BOURCIER
10/29/2013
10-28 tcon documentation

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 204629 **Applicant: Boehringer Ingelheim Pharmaceuticals, Inc.** **Stamp Date: March 5th 2013**

Drug Name: Empagliflozin/BI 10773 XX **NDA Type: 505(b)1**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		Oral dose administration (gavage for animal studies and tablets in the clinic)
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		Pharmacology/Toxicology labeling is in accordance with 21CFR.201.57. Human dose equivalents are expressed as MRHD multiples.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Impurities above the qualification threshold ([redacted]%) were qualified in nonclinical studies as per ICH Q3A(R2).
11	Has the applicant addressed any abuse potential issues in the submission?		X	Empagliflozin abuse potential studies were not carried out.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable. Empagliflozin is a new molecular entity that will not be marketed OTC.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __YES___X___

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MUKESH SUMMAN
04/26/2013

TODD M BOURCIER
04/26/2013
pharm/tox supports filing